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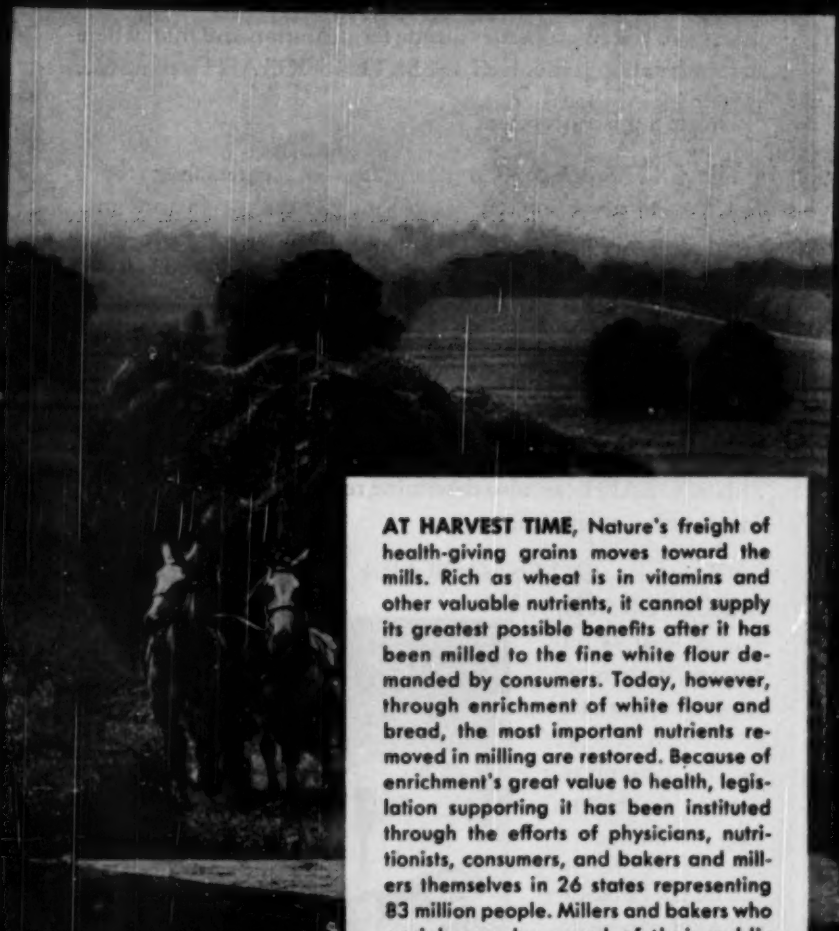

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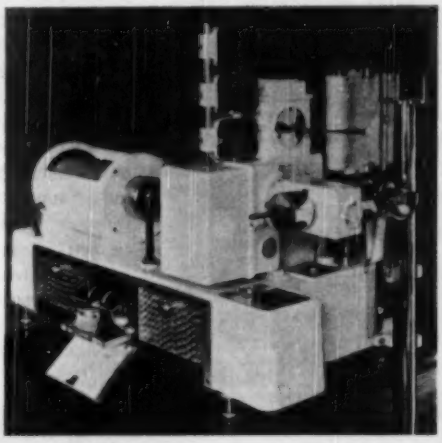

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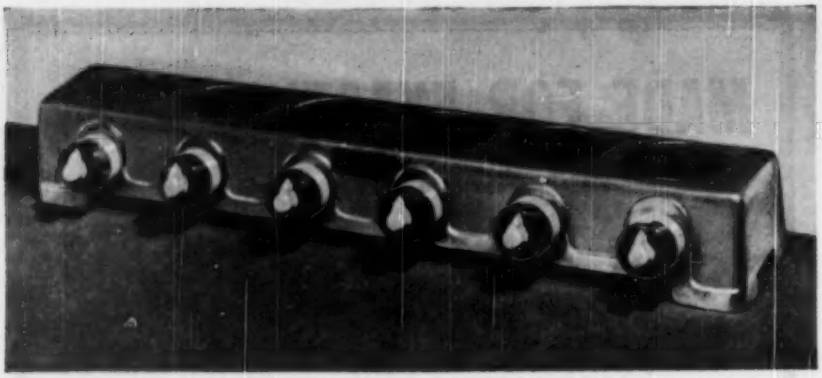

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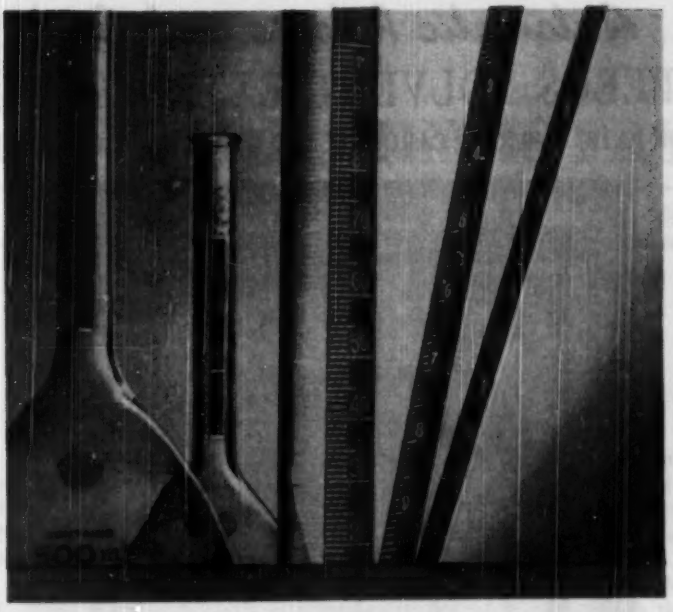

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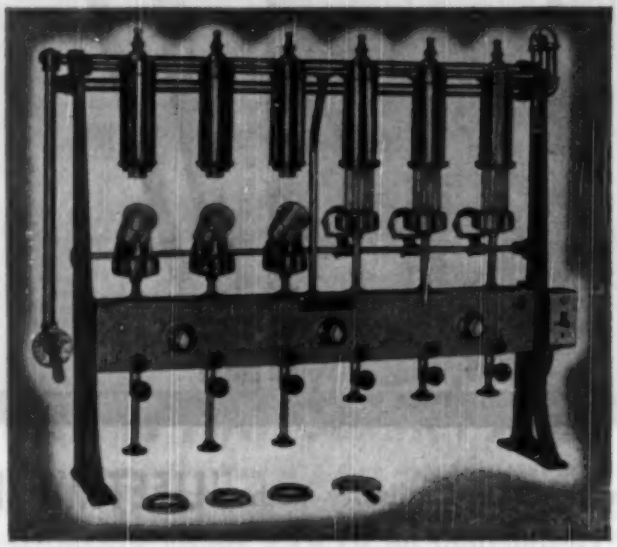

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CEREAL CHEMISTRY

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BETA-AMYLASE ACTIVITIES OF BARLEY, WHEAT, AND RYE [1]

VICTOR L. ERLICH [2] and GEORGE M. BURKERT [3]

ABSTRACT

Three fractions of beta-amylase are thought to be present in ungerminated barley, wheat and rye, the common cereals of the botanical tribe Hordeeae. One is water-soluble, the second is soluble in dilute salt solutions, and the third is made available by the action of proteolytic enzymes, such as papain.

Studies of the comparative amounts of these fractions were made, chiefly on barley and wheat, and were supplemented with data from the literature. Ungerminated grain, germinating grain, and malt were each extracted at 20°C. with water, salt solution, and papain solution respectively, for from two hours to three days, and the beta-amylase activity of the extracts determined.

Maximum beta-amylase activity of the water extract of the ungerminated grains was obtained within two to three days, and more quickly when the grains were germinated. When limited amounts of salts were added to the infusion, the activity of the extract increased. The amount of beta-amylase made available by such salt additions decreased as extraction progressed, and was negligible when extraction was completed. It also diminished as germination progressed. The maximum beta-amylase values to be reached in extraction were therefore not increased upon salt additions. The differential effects of salt additions were correlated with the ash contents of the cereals studied. It is concluded that the water-soluble and the salt-soluble activities constitute the "active" beta-amylase fraction, its water-soluble portion being the one which is made available by the water solution of salts contained in the grain.

The "inactive" fraction of the "total" beta-amylase activity is rendered water-soluble by proteolytic enzymes. This fraction was little affected by time and temperature of extraction, but decreased during germination while the "active" fraction increased.

The beta-amylase activity of a cereal malt is generally assumed to be preëxistent in the mature, ungerminated grain. Only a part of this activity, however, is readily available, or "free," in the water

[1] Manuscript received December 21, 1949.
[2] 890 Park Avenue, New York, New York.
[3] Wisconsin Malting Company, Manitowoc, Wisconsin.

extract of an ungerminated cereal. The presence of substantial fractions of inactive beta-amylase appears to be common in wheat, barley, and rye (8, 21). While all cereals belong, botanically, to the Gramineae, or grass family, these three are in the tribe called Hordeeae which does not include the other common cereals (18).

There is some controversy as to how the inactive portion of amylase is made available (11). Dull and Swanson (11) have suggested the term "bound" to designate the amylase fraction which is inactive due to physical causes, and "latent" for that rendered active by chemical reaction. Their opinion was that bound beta-amylase is released by salts from parts of the protein matrix, where it is adsorbed or occluded.

The use of papain in analytical methods for determining "total" beta-amylase activity is based on the fact that the latent fraction of the enzyme is made available by proteolytic enzymes. It is controversial whether this activation is the direct or indirect result of proteolysis, a reduction reaction involving the sulhydryl groups of the enzyme molecules (39), or results from the removal of inhibiting substances (19).

The present investigation was undertaken to secure further information concerning the comparative amounts of water-soluble, salt-soluble, and latent fractions of beta-amylase in ungerminated barley and wheat, in the germinating grains and in malts prepared from them.

Materials and Methods

Samples of different types of barley, wheat, and rye were selected for the evaluation of the beta-amylase activities of the ungerminated grains, of these grains during germination, and of the final malts. The barleys were all of the six-rowed spring type used for commercial malting. Each sample lot of barley was designated by a serial number according to the origin, viz: North Dakota B-1 to B-11, South Dakota B-21 to B-25, Minnesota B-31 to B-38, and Montana B-41 to B-42. The wheats, W1 to W3, represented three different hard winter wheats; the rye samples were labeled R.

Ten grams of the conventionally ground samples of grain or malt were infused with 200 ml. distilled water or salt solution, with or without addition of two grams papain (Merck). The extractions were conducted at 20°C. over periods of 2, 22, 46, or 70 hours, and at 30°C. for 2 hours. Toluene was added to all extractions of more than 3 hours. Extractions at 40°C. were made in some instances but are not reported in this study because destructive effects on the beta-amylase activities became apparent, particularly in the presence of salts and of papain (3, 15).

The beta-amylase activities of the ungerminated grains were measured in the filtered extracts according to the conventional method for determining the diastatic power (Cereal Laboratory Methods, 5th ed., 1947). Alpha-amylase activities were disregarded because the corresponding saccharifying activities were below the margin of analytical error of the diastatic power evaluation, even in the presence of salts or papain.

For the germinated wet grains, which were dried *in vacuo*, and for the kiln-dried malts, both the diastatic power or total saccharifying activity and the alpha-dextrinizing activity were measured in each extract. The latter determination was by the method of Olson, Evans, and Dickson (29), but the equivalent saccharifying activities were averaged from the values given by these authors and those published by Ehrnst and Lucht (13), which differ somewhat in the lower and higher brackets of activities. The beta-amylase activities were calculated as the difference between the total and the alpha-saccharifying activities, and expressed in terms of maltose equivalent divided by 4 (°L.).

Results

Influence of Salt- and Papain-Concentrations on the Availability of Beta-Amylase from Ungerminated Grains. It has been reported for wheat (10, 20) and for barley (11) that the amount of beta-amylase extracted increases with the concentration of neutral salt present, but reaches a maximum at approximately 0.1 to 0.2 *N* salt concentration. In these experiments the salt used amounted to about 12 to 15% of the weight of the grain. Salts present in greater amounts may exert detrimental effects either by depressing the activity of the solution (14) or by acting as precipitating ("salting out") agents (30, 31).

When increasing quantities of proteolytic enzymes such as papain, trypsin, rennet (7, 8) or ficin (3) are added to the water infusions of the grains, increasing beta-amylase activities result in the extract, until the highest activity is reached in a given time of extraction. Twenty parts of commercial papain per hundred parts of grain are usually more than the quantity necessary to give solutions of maximum activity (10), but may exert slightly detrimental effects over longer periods of extraction, especially at higher temperatures (3).

The complementary effect of salt and papain is illustrated in Table I by values obtained on two very similar samples of hard wheat for which the influence of salt alone was pronounced. The data for the suboptimal papain concentrations are reproduced from the paper of Jozsa and Gore (20).

The salt effect exceeded that of papain at the low, suboptimal concentrations of the latter. Increasing papain quantities reduced this

difference and then progressively exceeded the activating influence of the salt until the highest activation of enzyme by papain was attained. The complementary effect of salt plus papain was pronounced at the lowest papain concentration compared with that of papain alone, but it diminished with increasing papain activation and disappeared as soon as the papain quantity became sufficient for maximum activation. Over prolonged periods of extraction, addition of salt to the papain-grain infusion resulted in lower beta-amylase values than with papain alone.

Influence of Time of Infusion on the Water-, Salt-, and Papain-Soluble Beta-amylase Activities of Ungerminated Grains. Results obtained in the extraction of several barley samples with distilled water

TABLE I

COMBINED INFLUENCE OF SALT AND PAPAIN ON THE AVAILABILITY OF BETA-AMYLASE IN EXTRACTING HARD WHEAT

Duration of Extraction at 20°C.	Extraction-Medium					Differential Effects		
	Water Distd.	Salt without Papain	Papain			Papain compared with Salt	Papain+Salt compared with	
			Ratio to Wheat	Without Salt	Plus Salt		Salt Alone	Papain Alone
Hours	°L.	°L.	%	°L.	°L.	°L.	°L.	°L.
2[1]	44	102	0.01	48	103	−54	+ 1	+55
2[1]	44	102	0.02	58	105	−44	+ 3	+47
2[1]	44	102	0.03	72	115	−30	+13	+43
2[1]	44	102	0.10	90	120	−12	+18	+30
2[1]	44	102	0.25	119	147	+17	+45	+28
2	38	98[2]	20	176	178	+78	+80	+ 2
22	50	123[2]	20	198	196	+75	+73	− 2
46	62	128[2]	20	203	195	+75	+67	− 8

[1] Extraction results taken from the paper of Jozsa and Gore (20) with the kind permission of the publishers of Industrial and Engineering Chemistry; 0.03 *N* NaCl or 2% of wheat meal in 1:10 suspension.

[2] 0.1 *N* NaCl or 12% of wheat meal in 1:20 suspension (Wheat W-1).

without and in the presence of either salt or papain are reported in Table II. The figures are representative of similar experiments made on a greater number of different sample lots of spring barleys. Results reproduced from the paper of Davidson (9) are added because they refer to winter barleys of low diastatic power, treated under conditions of grain and salt concentrations which differed from those employed in our experiments.

The water-, salt-, and "total" papain-soluble activities of the spring barleys increased greatly during the first day of infusion at 20°C., they continued to rise somewhat to a maximum during a second, but showed practically no change on a third day. This is in agreement with similar

experiments reported in the literature (3, 27). Besides sample differences, the fineness of the grain meal influence such results (4). With Davidson's winter barleys the increases of the activities with time of extraction were less pronounced.

The activating effect of salt additions to infusions of spring barleys was small during the first hours of the extraction, and nearly disappeared in the longer extractions. Sodium and potassium chlorides,

TABLE II

INFLUENCE OF DURATION OF INFUSION (AT 20°C.) ON THE AVAILABILITY OF BETA-AMYLASE IN WATER-, SALT-, AND PAPAIN-EXTRACTIONS OF BARLEY GRAIN

Barley Sample	2 Hours			1 Day			2 Days			3 Days		
	Distd. Water	Salt	Papain	Distd. Water	Salt	Papain	Distd. Water	Salt	Papain	Distd. Water	Salt	Papain
	°L.	°L.	°L.	°L.	°L.	°L.	°L.	°L.	°L.	°L.	°L.	°L.
I[1]												
B-1	57	59	111	116	114	161	—	—	—	—	—	—
B-3	69	76	120	111	116	160	123	126	168	—	—	—
B-6	80	84	130	122	130	175	133	136	180	—	—	—
B-21	69	76	125	108	109	156	—	—	—	—	—	—
B-23	86	89	146	129	127	178	—	—	—	—	—	—
B-25	93	—	159	153	—	218	176	—	223	—	—	—
B-31	65	70	113	112	114	149	120	121	154	—	—	—
B-41	101	108	209	156	158	249	178	180	268	—	—	—
B-11	88	95	160	143	148	202	150	156	206	149	155	206
B-11	88	98*	160	143	144*	202	150	149*	206	149	151*	206
II[2]												
B-11	87	93	147	141	152	207	150	156	206	153	163	206
B-11	87	94*	147	141	144*	207	150	155*	206	153	152*	206
III[3]												
B-a	31	—	59	36	—	62	48	—	74	49	—	74
B-b	41	25	71	47	29	75	—	—	—	57	33	86

* Figures marked with asterisk refer to extractions in presence of KCl, all others to those in presence of NaCl.

[1] Spring barleys; grain infusion 1 part to 20 parts of water; salt in 0.1 *N* solution equivalent to 12% of the grain; papain equivalent to 20% of the weight of the grain.

[2] Spring barley; grain infusion 1 part to 30 parts water; salt in 0.17 *N* solution equivalent to 30% of the weight of the grain; papain added in amount equivalent to 15% of the weight of the grain.

[3] Winter barleys; extraction results reproduced from the paper of Davidson (9), obtained under infusion conditions identical to those described in footnote 2 (above).

compared on equimolar bases, gave similar results, but some significant differential salt effects on the availability of beta-amylase have been reported (11)[4]. According to Davidson's reports, salt additions resulted in lower beta-amylase values in the case of winter barleys. The lower values were not due to the difference in the extraction conditions, as shown by comparative experiments conducted with spring barleys, given in Table II under heading II.

[4] See Discussion. Sodium-bicarbonate was found to give extraction results similar to but always slightly higher than those obtained with sodium chloride; this is, however, in line with the fact that bicarbonate ion has a stimulating effect upon the beta-amylase activity in water solution (14).

The beta-amylase activities of wheat show trends which are basically similar to those described for barley. In the case of wheat also, the type of grain greatly influences the beta-amylase activity (9, 20, 33, 34). Extraction results for some examples of wheat and rye are given in Table III.

In these examples, the rate at which the water-soluble fraction became available was slow compared with that of the spring barleys, and solubilization still continued after two days of infusion at 20°C.

The salt additions, however, increased the extraction of the beta-amylase to a much higher degree than for barley in the initial periods of the infusion, but the salt effect diminished and finally disappeared

TABLE III

INFLUENCE OF DURATION OF INFUSION (AT 20°C.) ON THE AVAILABILITY OF BETA-AMYLASE IN WATER-, SALT-, AND PAPAIN-EXTRACTIONS OF WHEAT AND RYE GRAINS

Grain Sample	2[1] or 3[2] Hours			1 Day			2 Days			3 Days		
	Distd. Water	NaCl	Papain	Distd. Water	NaCl	Papain	Distd. Water	NaCl	Papain	Distd. Water	NaCl	Papain
	°L.	°L.	°L.	°L.	°L.	°L.	°L.	°L.	°L.	°L.	°L.	°L.
Wheat												
W-1[1]	38	98	176	50	123	198	62	128	203	—	—	—
W-3[1]	53	91	151	64	131	185	135	141	188	150	141	185
W-a[2]	45	—	90	44	—	90	45	—	89	47	—	87
W-b[2]	54	75	104	54	80	102	59	—	103	61	78	103
Rye												
R-1[1]	66	71	94	78	79	96	79	82	94	81	83	96
R-a[2]	66	—	85	71	—	89	77	—	90	83	—	94
R-b[2]	89	84	111	96	92	112	102	—	114	102	93	109

[1] Grain infusion 1 part to 20 parts of water; salt in 0.1 *N* solution equivalent to 12% of the grain; papain added in amount equivalent to 20% of the weight of the grain.
[2] Extraction results reproduced from the paper of Davidson (9); Infusions of 1 part grain to 33 parts water; salt in 0.17 *N* solution equivalent to 30% of the weight of the grain; papain added in amount equivalent to 15% of the weight of the grain.

when the extraction was sufficiently prolonged to give the highest level of the water-soluble activity. In the latter case, the added salt sometimes had a slightly depressing action.

The effects on rye are similar in principle, and are also subject to differences dependent upon the type of grain (7, 9, 21).

The papain-activated beta-amylase fractions, calculated as the difference between the "total" and the respective water- and salt-soluble activities, are recorded in Fig. 1, for three samples of barley with widely different diastatic activities, for one hard wheat and one rye.

The water-insoluble fractions of barley and rye decreased pronouncedly during the first day of extraction, but to a lesser degree on the following days. The corresponding effects on wheat were quite

inconsistent during the initial periods of infusion; strong decrease of this fraction occurred, however, with progressing-extraction. Calculation of these papain-activated fractions from data found in previous publications confirms these findings.

The salt-insoluble fractions were much less affected by the time of extraction; they decreased but slightly in the initial periods and still less with more complete extractions. The similarity observed in this respect for barley and rye as well as for wheat appears to be significant.

Experiments conducted comparatively at 20°C. and at 30°C. showed the influence of higher extraction temperature to be in accordance with that of increased duration at the same temperature.

Influence of Germination on the Beta-Amylase Activity of Barley and Wheat. The "free" beta-amylase activity, as determined conventionally in two to three hour infusions, increases greatly during the

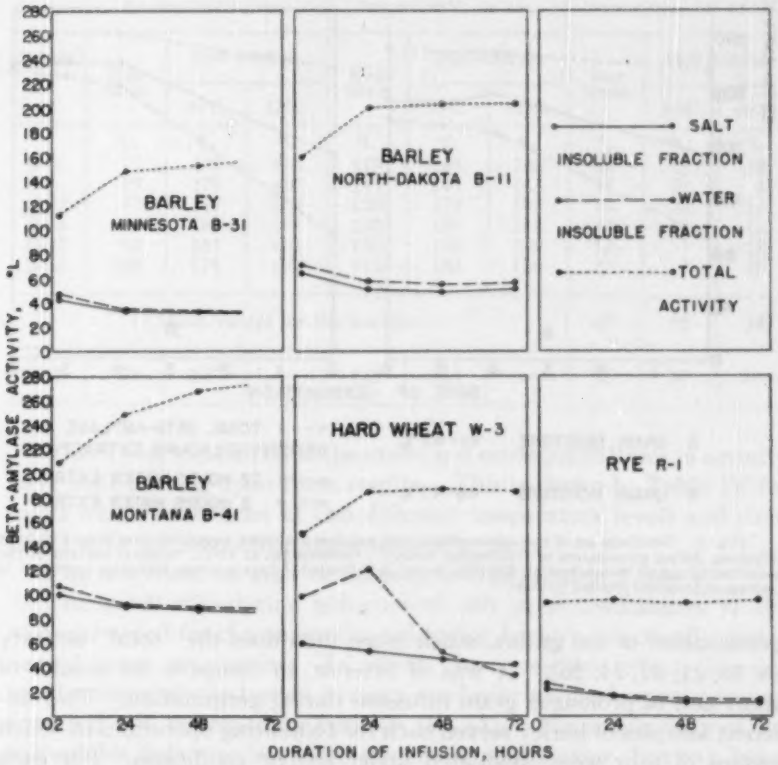

FIG. 1. Effect of the time of infusion on the water- and salt-soluble beta-amylase fractions as activated by papain in ungerminated barley, wheat, and rye. Infusion at 20°C. of one part grain for 20 parts water; salt effect of 0.1 *N* sodium chloride. Beta-amylase activities expressed in maltose-equivalents divided by four.

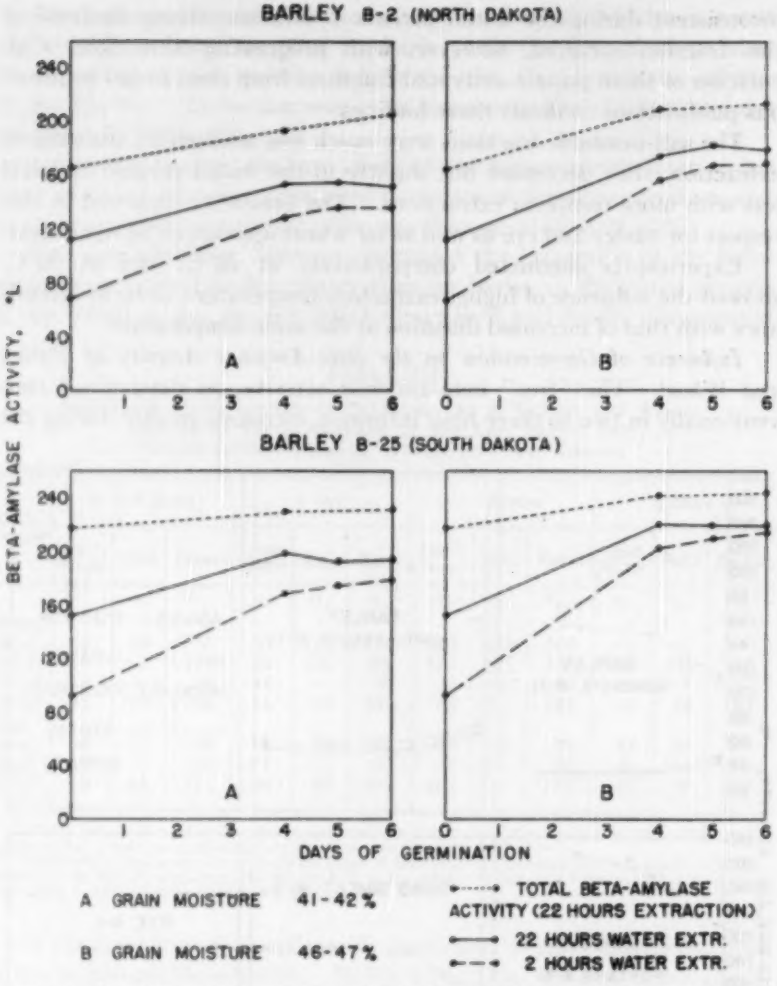

FIG. 2. Development of the water-soluble beta-amylase activities, measured after 2- and 22-hour infusions, during germination of commercial barleys. Germination at 16°C., moisture content of the germinating grains maintained at different levels, as indicated. Beta-amylase activities expressed in maltose-equivalents divided by four.

germination of the grains, much more than does the "total" activity (6, 16, 21, 22, 24, 26). It was of interest to compare the results of short and of prolonged grain infusions during germination. Two different samples of barley served each for 16 malting operations in which groups of four were conducted under similar conditions. For each group, the grain moisture was maintained on a different level, constant throughout the germination. The mean values obtained for the two

barleys on two of these groups of subsamples after four, five and six days of germination, are shown in Fig. 2.

The influence of duration of extraction upon the availability of water-soluble beta-amylase diminishes greatly with increasing length of germination, i.e., with the disaggregation of the inner kernel structure. Therefore, the "free" activity measured after 22-hour infusion increases during germination less than does the conventionally determined beta-amylase activity. The same is true for the "total" activity. A 22-hour infusion at 20°C, is practically sufficient for full availability of the free and the total beta-amylase activities of commercial barley malts.

TABLE IV

INFLUENCE OF THE DURATION OF INFUSION ON THE AVAILABILITY OF THE WATER-SOLUBLE BETA-AMYLASE ACTIVITIES OF KILN-DRIED MALTS

Sample	Infusion 20°C., 2 hrs.			Infusion 20°C., 22 hrs.			Increase from 2 to 22 hrs.		
	Raw Grain	Malt dried at:		Raw Grain	Malt dried at:		Raw Grain	Malt dried at:	
		50°C.	72°C.		50°C.	72°C.		50°C.	72°C.
	°L.	°L.	°L.	°L.	°L.	°L.	°L.	°L.	°L.
B-5	77	139	116	119	154	134	42	15	18
B-8	99	179	140	141	201	143	42	22	3
B-22	77	149	108	128	171	127	51	22	19
B-24	90	167	131	130	185	144	40	18	13
B-32	83	161	114	136	168	132	53	7	18
B-35	100	176	139	143	183	154	43	7	15
Mean values for the barleys							45	15	14
W-1	98	127	—	123	143	—	25	16	—

The drying conditions of the malts and consequent losses in activity have little bearing upon these results. This is shown in Table IV for malts which were dried at two different temperature levels and compared with the respective ungerminated grains.

The salt effect on malt extractions is illustrated in Table V.

The small stimulating influence of salt upon availability of the enzyme, noted for the ungerminated spring barleys, practically disappeared upon germination. In wheat, the strong salt effect observed for the ungerminated grain became markedly reduced upon germination, but still remained appreciable (14, 15). The availability of the salt-soluble beta-amylase increases during germination, but to a lesser degree than that of the respective water-soluble fractions.

The papain-activated fractions of beta-amylase in both barley and

wheat malts, little affected by the conditions of infusion, were appreciably lower than in the respective ungerminated grains. Some examples are given in Table VI for commercial malts of 5 to 6 days germination.

Comparisons of the activities were based upon measurements obtained in two-day extractions of the ungerminated grains and one-day extractions of the malts. The papain-activated fractions decreased progressively in amount during germination; this is illustrated by sample B-2 which compares three stages of development of green malt. In commercial malts substantial amounts of the papain-activated

TABLE V

INFLUENCE OF SALT ADDITIONS ON THE BETA-AMYLASE ACTIVITIES IN THE EXTRACTION OF MALTS

Sample	Infusion 20°C., 2 hrs.			Infusion 30°C., 2 hrs.			Infusion 20°C., 22 hrs.		
	Water	NaCl	Increase	Water	NaCl	Increase	Water	NaCl	Increase
	°L.	°L.	°L.	°L.	°L.	°L.	°L.	°L.	°L.
B-9	109	111	2	104	110	6	—	—	—
B-10	165	165	0	169	170	1	176	176	0
B-26	82	84	2	80	83	3	—	—	—
B-32	114	115	1	115	114	− 1	132	129	− 3
B-36	118	117	− 1	119	120	1	124	125	1
B-37	114	116	2	—	—	—	122	122	0
B-38	105	105	0	—	—	—	116	116	0
Mean values of increases (on malts)			1			2			0
Mean values of increases (on raw grains)			5[1]			3			2[1]
W-1	82	127	45	85	137	52	110	143	33
Increase on raw grain:			60[2]						73[2]
W-2	46	78	32	56	80	24	—	—	—

[1] See Table II.
[2] See Table III.

fraction of the enzyme are present, but when germination is continued beyond usual practice, the beta-amylase becomes completely water-soluble as shown by previous workers for wheat (22) and for barley (38)[3].

The "total" beta-amylase activity of commercial green malt is always higher than that of the respective ungerminated grain, compared on a weight basis. Losses in beta-amylase activity become noticeable after prolonged periods of germination (22), but are unusual in normal malting practice. The increases reported in Table VI can be

[3] Weichherz and Aamus (38) found the "total" diastatic power remaining slightly higher than the "free" activity, but they disregarded the papain effect upon the alpha-amylase activity (14).

TABLE VI

COMPARISON OF BETA-AMYLASE ACTIVITIES OF MALTS AND OF THE RESPECTIVE RAW GRAINS

Sample	Salt-Soluble Fractions[1]		Total Activities[2]			Papain-Activated Fractions		
	Raw Grain	Green Malt	Raw Grain	Green Malt	Kilned Malt[3]	Raw Grain	Green Malt	Kilned Malt
	°L.	°L.	°L.	°L.	°L.	°L.	°L.	°L.
B-2	122	153	168	195	—	46	42	—
B-2	122	177	168	209	—	46	32	—
B-2	122	186	168	215	—	46	29	—
B-3	126	—	168	—	158*	42	—	30
B-4	136	—	193	(210)[4]	194	57	—	37
B-5	132	174	180	194	154*	48	20	14
B-6	136	—	180	—	167*	44	—	26
B-7	149	186	191	207	202	48	21	12
B-8	154	206	213	227	170*	59	21	27
B-22	141	172	194	195	132*	53	23	5
B-24	143	176	182	211	160*	39	35	16
B-25	178	223	223	244	—	45	21	—
B-32	149	182	205	215	148*	56	33	16
B-34	136	179	177	193	186	41	14	11
B-35	156	196	202	222	177*	46	26	23
B-42	203	—	286	(310)[4]	294	83	—	63
W-1	128	(159)[4]	203	(219)[4]	203	75	(60)[4]	60

[1] Activities measured after 46 hours infusion with 0.1 *N* NaCl solution for the raw grains, after 22 hours for the malts.
[2] Total activities extracted in presence of 20% papain of grain and malt; 46 hours for grains, 22 hours for malts.
[3] The malts marked with an asterisk (*) are brewers, dried at final temperatures of 72°C., the others were dried at 50°C.
[4] Values are estimated.

explained by the fact that 6 to 9% of the dry matter is lost during the malting process; in a few cases the difference was found to be somewhat greater, but it must be considered that infusion of the ungerminated grain over periods of two days may not always give complete extraction and infusions continued beyond that duration may give low results, especially in presence of salts and of papain.

Discussion

Intervarietal correlation between the total saccharifying power of barley and the salt-solubility of the protein fractions has been shown to exist (1). Increase in availability of the beta-amylase of barley by infusion with solutions of salts of various anions and in different concentrations is of a nature similar to such salt effects on the peptization of the grain proteins (11, 36).

It is known that "free" beta-amylase increases with the concentration of the flour mashes (20), it is higher in whole wheat than in patent flour, and still greater in the low-grade flour portions, parallel with the increasing ash contents (5, 9, 37). Likewise the degree of peptization

of different wheat flours has been shown to be positively correlated with their ash contents (32), as are also the proteolytic activities (17). The availability of the latter in grain infusions is apparently also increased in the presence of salts (12, 23).

Similar differences in the water-solubility of beta-amylase should become apparent when different grain types are compared with regard to their ash content. Barley, for instance, may vary in its ash on a dry basis from 2.4 to 3.5% (Spring barleys have usually 2.5 to 3.0% ash, Winter varieties up to 3.5%). Wheat has normally less than 2% ash (hard varieties 1.4 to 2.1%); the ash of rye is intermediate between barley and wheat.

According to the experiments reported here, salts only slightly increase the beta-amylase activity of spring barleys and of rye, but effect a considerable increase in the case of hard wheats. Davidson (9), reported the beta-amylase activity of winter barleys pronouncedly depressed, that of rye only slightly, and that of wheat increased. This could mean that the soluble ash of the barleys and ryes was sufficient to release nearly all or all of the "bound" beta-amylase, whereas the wheats needed substantial supplementation by added salts to complete the liberation, unless the time of extraction was considerably extended.

As reported in the present study, prolonged infusion of grains and malts in distilled water led to increasing beta-amylase activity until a maximum was obtained. Salts added in limited amounts accelerated this solubilization more or less, but the maximum availability remained the same as could be reached in sufficiently long water infusions.

In the malting process, the soluble minerals apparently diffuse into the core of the kernel during the soaking of the grain, and can be expected to act upon bound beta-amylase similarily, though more slowly, than they do in the water infusions of the grain meals. This may be one of the reasons why beta-amylase is more readily made available by water as germination progresses. Similarly, the accelerating effect of salts noted in the infusions of the ungerminated grains, is reduced or disappears upon their germination.

Therefore, it seems unwarranted to make a fundamental distinction between water-soluble, or "free" beta-amylase, and the "bound" or salt-soluble enzyme. The conclusion is suggested that both portions are "active" though not immediately available in their entirety because adsorbed, enveloped, or otherwise physically bound to certain fractions of the protein matrix. This "active" beta-amylase is completely released upon action of salts in solution furnished partly or fully by the grain ash. Such release proceeds simultaneously with peptization of the protein complexes, which is not necessarily a protein hydrolysis (35).

The "inactive" or "latent" beta-amylase fraction should be considered as that portion of the "total" activity which can not be made available by water in the presence of adequate salt quantities. The "inactive" beta-amylase is present either in reversibly oxidized form or chemically bound to another protein molecule. It is rendered water-soluble by proteolytic enzymes acting either as such or as reducing agents involving the sulfhydryl groups, or both.

It is here reported that these "inactive" fractions are little affected when time or temperature of the grain infusions are increased. In fact, they diminish slightly under such conditions, probably due to proteolytic action of the grain enzymes which are present. Curves plotted for the "total" activities against time of infusion show nearly the same slope as those of the respective salt-soluble "active" fractions and the increasing availability of the latter determines predominantly that of the "total" papain-soluble activities. Salts added to the papain-water infusions have either no effect or a detrimental effect. Commercial papain preparations contain enough soluble mineral salts to supply the grain-water infusions with the necessary salt supplements (14). Heat-inactivated papain has been reported to increase the water-extracted beta-amylase fraction of barley (7). Myrbäck and Myrbäck (28) did not confirm this effect but this is not necessarily a contradiction; they measured after a 20-hour infusion at 30°C., thus after the activity had been already completely released with water, whereas Chrzaszcz (7) compared the activities after 14 hours at 20°C; see above Table II. Papain thus provides for the release of both the "active" and the "inactive" beta-amylase.

During germination the proteolytic activities of the cereals are known to increase greatly (2, 25), and this may cause the progressive decrease of the "inactive" beta-amylase fraction. The "active" fraction, in turn, increases correspondingly beyond that present in the ungerminated grains. This increase, however, is much smaller than that of the seemingly free activity measured on the basis of conventional water extractions.

It is of interest to note that these changes, which occur during germination, take a course opposite to that apparent during the ripening periods of the cereal grains, as shown for wheat by Schwimmer (34), who thought the progressive insolubilization of beta-amylase to be connected with the dehydration of the maturing grain and the formation of the final protein complexes.

The effects of salts and of papain on the availability of cereal alpha-amylase, discussed for barley and wheat malts by Erlich and Burkert (14), are apparently of a different nature than those on beta-amylase. However, when these activities are measured in their water extracts,

it has to be considered for both types of enzymes that any infusion of cereal grains or malts in distilled water is actually an extraction by a weak salt solution produced by the soluble ash minerals. This is particularly important when the release of the enzyme activity from the grain is highly sensitive to small changes in salt concentration. Such is the case for alpha-amylase of both barley and wheat malts and for beta-amylase of wheat grain and flour, of malted wheat flour and some commerical wheat malts, but to a lesser degree for ungerminated barley and rye; it is not the case for beta-amylase of barley malt.

The presence of salts and possibly other soluble compounds in commercial papain also influences the utilization of these preparations in analytical procedures. In the case of alpha-amylase, the action of papain in apparently due only to its soluble ash content, and the maximum effect is lower than that obtainable with adequate salt solutions. No chemical activation of an "inactive" alpha-amylase fraction can be observed, as common for the beta-amylase activities of wheat, barley and rye.

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POLYOXYETHYLENE MONOSTEARATE AS AN ANTI-STALING ADJUNCT IN BREAD [1,2]

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ABSTRACT

Various methods for determining the physical changes taking place during the staling of bread baked with and without the addition of polyoxyethylene monostearate have been evaluated. Measurements of the decrease of crumb thickness indicate that the use of this adjunct markedly retards the firming of bread as it ages from 4 to 115 hours.

The crumbliness test for staling, the Katz method for measuring the decrease in soluble starches, the swelling power test, and the farinograph method of measuring the decrease in water absorptive capacity give anomalous results when bread contains polyoxyethylene monostearate. This failure to give measurements indicative of the age of the bread is attributed to the polyoxyethylene monostearate having reduced the hydrophilic characteristics of the starch so that the normal molecular alignments of starch chains with each other and with water fail to occur.

The only traditional staling rate measurements which appear to be of value are those dealing with changes in softness and possibly in crumbliness, but crumbliness appears to be more indicative of tenderness than of age.

Katz (10), who did such extensive work on the problem of bread staling during the period 1912–1939, devised many of the tests for staling in use today. Most of these have been modified and revised to make use of present-day equipment, but their essential character has remained unchanged. Since 1930 several reviews of these staling tests and their modifications have appeared in *Cereal Chemistry*. Mention should be made especially of the reviews by Platt (11), Cathcart (4), Alsberg (1), and Geddes and Bice (9).

This paper reports the results of applying the traditional staling tests to bread containing polyoxyethylene monostearate (POEMS). The studies reported show the effect of POEMS on (a) changes in compressibility or, as Bice and Geddes (3) prefer "changes in the softness of bread crumb," (b) changes in crumbliness as a measure of staling, (c) changes in crumbliness as a measure of tenderness, (d) changes in water absorptive power as a measure of staling and (e) changes in soluble starch content as a measure of staling. In addition the effect of POEMS on the wetting rate of bread crumb is reported.

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[2] Contribution from C. J. Patterson Company, Kansas City, Missouri.

Changes in Compressibility

The most noticeable change taking place in bread as it becomes stale is the increase in firmness or the decrease in compressibility of the crumb.

Katz (10), Platt (11), Bailey (2), Combs (6), Straub (12), and many others have described compressimeters to be used in the measurement of staling rates. Two techniques are involved. Either the instrument determines the weight necessary to bring about a predetermined decrease in crumb thickness or it determines the decrease in thickness caused by addition of a given weight. In this paper the latter method is applied. In all instruments, the pressure is applied for a definite length of time. These methods have been discussed fully by Bice and Geddes (3).

For the various studies reported in this paper, all bread was baked by the same sponge-dough method, employing the following formula except where otherwise indicated:

Ingredient	*Per Cent*	*Weight (gms.)*
Flour	100	700 (14% moisture)
Water	57–69	399–483
Yeast	2.0	14.0
Yeast food	0.5	3.5
Milk	3.0	21.0
Sugar	5.0	35.0
Lard	2.0	14.0
Salt	2.0	14.0

A 60% sponge with an absorption of 57% was used. All the yeast was dispersed in the sponge water prior to the addition of the flour. In weighing sponge ingredients, yeast food and malt (when needed) were added directly on top of the flour.

The sponge was mixed one minute at low speed, then one minute in second speed. To prevent excessive flour dust, the water and yeast solutions were poured into the mixing bowl before the other sponge ingredients were added. The sponge was placed in a metal trough and fermented four and one-half hours at 84°F. (29°C.) and 85% relative humidity.

The dough was mixed as follows: Dry ingredients and the remaining water were placed in the bowl and mixed at the lowest speed for three minutes. During the first minute the sponge was added in three or four approximately equal lumps. After low-speed mixing, the dough was mixed at second speed for the predetermined time. The mixing bowl was jacketed with ice water and chilled water was used as the dough water so that the dough came from the mixer at 80°F. (26.6°C.).

Immediately after mixing, the dough was again placed in the fermentation trough and a thermometer was inserted. After one minute

the temperature was recorded and the trough was returned to the fermentation cabinet for 35 minutes. At the end of this period the dough was removed and two 380 gm. pieces scaled from it. Each of the pieces was formed into a ball by a manual operation which was intended to simulate the action of the commercial rounder. The pieces were then dusted and allowed to stand at room temperature on a dusted board for ten minutes.

After this period, which corresponds to the overhead proof time employed in the commercial bake shop, each piece was molded in a conventional commercial molder, placed in a pan, numbered and proofed in a proof box at 102°–104°F. (39°–40°C.) and 80% relative humidity until the top surface of the dough was ½ in. above the top of the pan.

The doughs were then placed in an oven and baked at 425°F. (218°C.) for 25 minutes. Baked loaves were placed on a rack and cooled to 100°F. (38°C.) before being doubly wrapped and heat-sealed in moisture proof waxed paper.

The compressimeter used in these studies consisted of a metal square, 2.5 in. by 2.5 in. and approximately 0.5 in. thick, which was attached rigidly at right angles to a round metal rod graduated in tenths of an inch. The combined plate and scale weighed 300 gm. In use, the rod was passed through a 2-in. glass tube which acted as a guide and on which was etched a reference mark. The instrument was adjusted before use by placing a 1-in. block under the metal plate and setting the reference mark to coincide with the 1-in. mark on the scale.

With the aid of a miter box, a slice of bread 1 in. thick was cut from the center portion of a loaf. The bottom crust was cut away and with the aid of a second miter box a 2-in. square was cut from the center of the slice. Any square containing large holes or a core was rejected. The sample was placed on a flat surface directly under the compressimeter plate and the plate was lowered until its face just touched the surface of the crumb. The zero reading representing the crumb thickness was taken from the scale. The compressimeter was released for a period of 60 sec., after which a second reading was made. The difference between the first and second readings corresponds to the decrease in crumb thickness. This difference multiplied by 100 and divided by the original thickness gave the per cent decrease in thickness of the crumb.

In all determinations of crumb compressibility, bread containing 0.5% polyoxyethylene monostearate (flour basis), the stearic acid ester of polyethylene glycol and hereafter designated as POEMS, was compared with bread containing no POEMS but otherwise baked at the same time by the same formula from the same flour under the same conditions.

Compressibility data taken during a 1¼ year period on a total of 179 samples of bread containing POEMS were averaged and the average compressibilities at 0.75, 1.75, 2.75, and 3.75 days are shown in Fig. 1. Several loaves, each containing a different sample of POEMS, were usually compared with a loaf containing a standard sample of POEMS and a loaf baked without the addition of POEMS. In all instances the POEMS was used at the 0.5% level. In all instances compressibilities were determined on three different days but not necessarily on consecutive days. The loaves were stored at room temperature, which, of course, varied from summer to winter and, to a lesser degree, from day to night.

All the curves in the literature have assumed the form of an equilateral hyperbola. The curves of Fig. 1 do not represent staling rates at any given temperature, but rather the average firming rates for

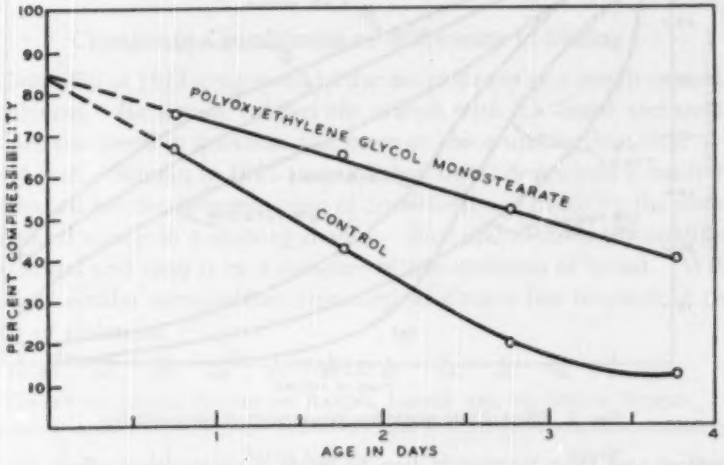

FIG. 1. The effect of age on compressibility.

bread containing POEMS and bread baked without the adjunct. The curve for the control bread has a steeper slope than that for the bread containing POEMS. Extrapolation of each curve to zero time indicates that both types of bread have the same initial softness.

Compressibility tests were also made on bread baked with 0%, 0.25%, 0.5%, 1.0%, and 2.0% POEMS. All bread was doubly wrapped in moisture-proof paper and stored at room temperature, approximately 70°–74°F. Starting when the loaves had been out of the oven four hours, compressibilities were determined at intervals until the bread was 115 hours old.

The crumb recoveries at the 4-hour period were not good, e.g., the

elastic limits had been exceeded. During the first eight hours after bread came from the oven, changes in bread firmness took place so rapidly that experimental errors in testing were no doubt very high. The results represented graphically in Fig. 2 show that the use of POEMS definitely changed the firming rate. Bread containing 0.5% POEMS had about the same compressibility at 77 hours that control bread had at 35 hours.

Effect of Increased Water on Compressibility. It appeared desirable to design experiments which would indicate whether the retarded firming of bread containing POEMS is caused by its reaction with the starch, thereby preventing water from being bound through hydrogen

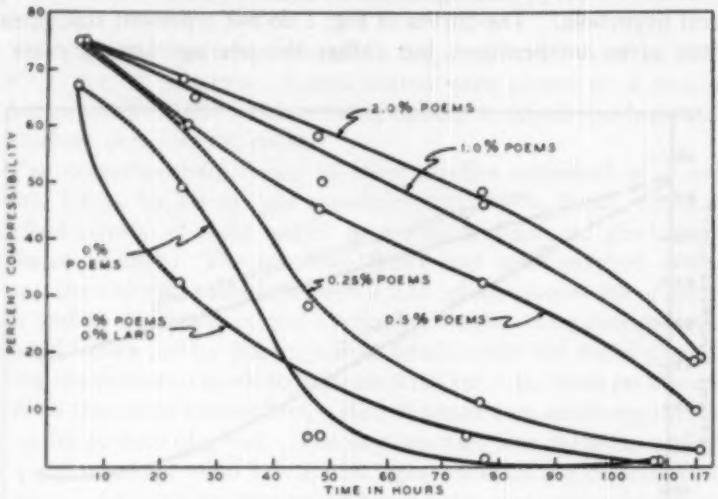

FIG. 2. Effect of polyoxyethylene monostearate on compressibility.

bonding and thus leaving it free to exert a plasticizing effect on the crumb. In other words, could the same retarded firming be brought about by the use of additional water in the dough, assuming that this water is retained in the baked bread?

Three series of loaves were baked with and without 0.5% POEMS. In each series water was increased in 2% increments until the dough was baked at an absorption 10% higher (16.6% higher based on water content) than the normal absorption. The two Kansas flours series baked were designated as A and B series, the Nebraska series as C. In the B series the dough at the maximum absorption was too slack to mould. Weights were recorded for total ingredients, dough, and baked bread. Compressibilities were determined in the manner previously indicated at 24, 48, and 72 hours.

Table I shows that the total per cent losses of volatile materials during fermentation and baking to be constant within one range of experimental error. Accordingly, the amount of water left in the finished bread is, within the limits of experimental error, roughly proportional to the amount of water added to the dough.

The compressibilities for the three series were so similar at each bread age that they were averaged. The resulting averages, plotted in Fig. 3, show that the additional water above the farinograph absorption retarded the firming process. However, with approximately 10% extra water, the bread baked without POEMS did not show the compressibility of bread containing 0.5% POEMS but with no extra water. It appears, therefore, that the mechanism involved when POEMS retards the firming of bread is not one involving simply the plasticizing effect of water. Also, there is no evidence from Table I that POEMS has any effect on moisture losses during baking.

Changes in Crumbliness as a Measure of Staling

Katz (10) in 1912 attempted to use crumbliness as a rough measure of staleness. He simply rubbed the crumb with his finger and noted whether the bread in question was more or less crumbly than that of a second loaf. Selman in 1943 (unpublished data) developed a mechanical method for the determination of crumbliness of cakes by the use of a standard screen in a shaking device. Bice and Geddes (3) modified the method and used it as a measure of the staleness of bread. With breads of similar composition, this method gives a fair measure of the degree of staleness.

TABLE I

EFFECT OF EXTRA WATER ON BAKING LOSSES AND ON BREAD SCORES

Poems (%)	Absorption* (%)	Baking Loss (%)			Score**		
		A	B	C	A	B	C
0	60	14.9	13.5	13.1	G−	G	G−
0	62	16.7	13.2	12.4	G−	G	G−
0	64	15.0	13.8	11.6	G−	G	G−
0	66	15.0	13.0	12.7	G−	G−	F+
0	68	16.0	12.4	15.2	F+	F+	F+
0	70	14.8		13.6	F	P	P+
0.5	60	15.2	13.3	13.5	G	G	G
0.5	62	16.9	15.8	14.5	G	G	G−
0.5	64	15.4	12.5	13.2	G	G	G
0.5	66	17.0	10.9	13.1	G	G−	G−
0.5	68	15.3	11.8	14.5	G−	G−	F
0.5	70	13.9	16.9	14.0	G−	F+	P

* Series C had absorption 0.5% higher than indicated by table.
** Scores are average for three consecutive days. G = Good; F = Fair; P = Poor.

With the aid of a miter box, three slices, each 1 in. thick, were cut from the center portion of the loaf. Using a second miter box, four 1-in. cubes were cut from the center of each slice. The twelve 1-in. cubes were weighed, placed in a No. 4 standard sieve and shaken in a Ro-Tap machine for 30 minutes at room temperature. The "throughs" were weighed and crumbliness was calculated as the weight of crumbs obtained from 100 gm. of bread. Before determining the crumbliness on the third day the remaining wrapped loaves were heated at 200°F. (93.3°C). for twenty minutes. Two hours later, crumbliness was determined for each of the loaves.

Figure 4 shows the increase in crumbliness for loaves from five doughs containing from 0% to 2.0% POEMS. If the degree of crumbliness is taken as a measure of staleness, bread containing 2% POEMS is more stale after six hours out of the oven than the control bread after 48 hours. Reheating the bread gave, as indicated by the dotted lines in Fig. 4, slight changes in crumbliness but did not restore the original crumbliness. Three of the five loaves showed an *increase*

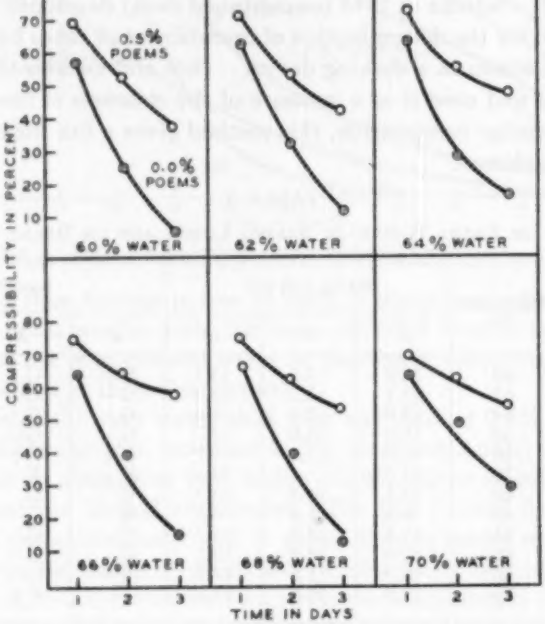

FIG. 3. Relation of water and polyoxyethylene monostearate to crumb compressibility.

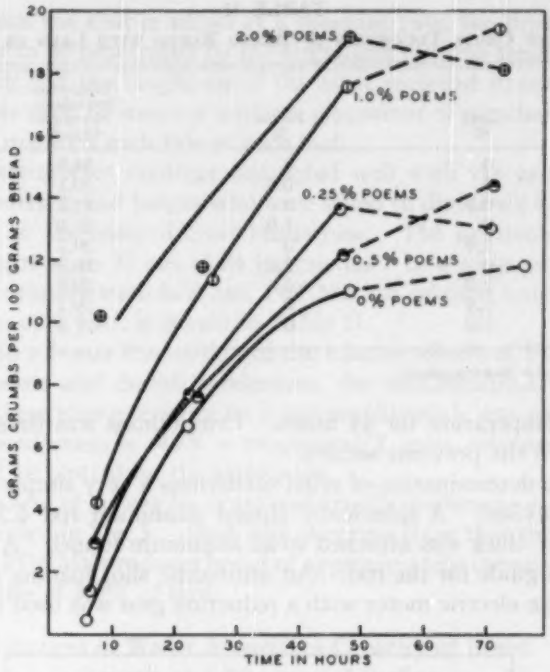

FIG. 4. Effect of polyoxyethylene monostearate on retardation of crumbliness in bread.

in crumbliness. The crumbliness of the five loaves appeared to increase in approximate proportion to the content of POEMS. The results cast, at least, a reasonable doubt on the value of the test as a measure of staleness.

Changes in Crumbliness as a Measure of Tenderness

All loaves tested in the previous section contained 2% lard. Since crumbliness did not appear to be a valid test for staleness, it was thought that it might be used as a measure of tenderness. Bread was baked by the method indicated earlier except that in one series of loaves, lard was used at 0.5%, 1.0%, 1.5%, and 2.0% levels without POEMS. In the second series POEMS was used at the corresponding levels without lard. Duplicate series were baked for determination of the relative crust tenderness by the penetrometer method described below; in these series, lard was used at levels up to 3.0% in 0.5% increments. Previous experiments had indicated that penetrometer tests were least erratic when carried out with bread approximately 44 hours old; so all loaves were doubly wrapped and heat-sealed in waxed paper and stored

TABLE II

RELATIVE CRUST TOUGHNESS OF BREAD BAKED WITH LARD OR POEMS

Lard %	Poems %	Toughness Seconds to Penetrate Crust*
0	0	38.9
0	0.5	20.1
0.5	0	31.4
0	1.0	21.0
1.0	0	29.7
0	2.0	16.8
2.0	0	29.9
2.5	0	29.7
3.0	0	27.3

* Average of 6 determinations.

at room temperature for 44 hours. Crumbliness was determined as indicated in the previous section.

For the determination of crust tenderness a very simple penetrometer was devised. A spherically tipped aluminum rod 6.25 in. long and 0.25 in. thick was attached to an aluminum funnel. A glass tube serves as a guide for the rod. An automatic shot loading device operated by an electric motor with a reduction gear was used to load the

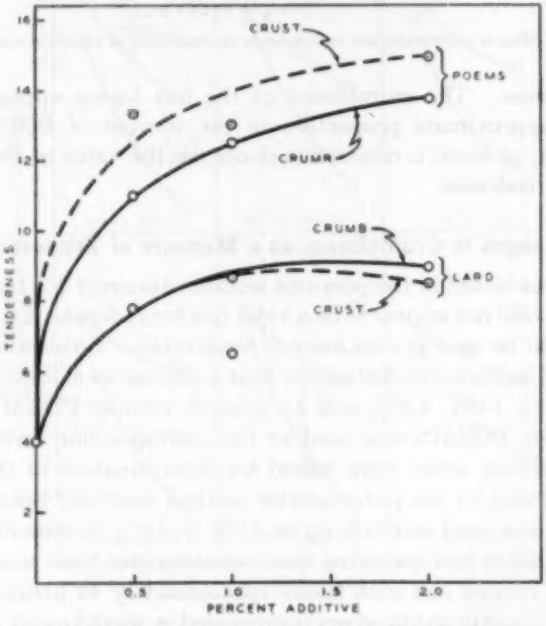

FIG. 5. Effect of hydrophobic additives on tenderness.

funnel. Since the shot is added at a constant rate, the time required for the crust to be penetrated by the aluminum rod was measured with a stop watch and the toughness of the crust recorded in seconds. A template was used to insure a uniform placement of punches, three of which were made on each side of each loaf.

The penetrometer readings correlated well with the estimates of four experienced bread judges who were asked to divide six loaves into two groups on the basis of crust tenderness. The penetrometer and the judges agreed on 22 out of 24 judgments. In a series of loaves in which the variables were lard and POEMS the relative toughness determined for each loaf, is shown in Table II.

To obtain a better conception of the relative effects of POEMS on crust toughness and crumb tenderness, the mathematical device of expressing these characteristics on a comparable scale was used. The equation: tenderness = (46.9 − toughness)/2 gave arbitrary values which could be plotted on the same scale.

The two sets of curves for crust and crumb tenderness as obtained above are shown in Fig. 5. Both sets of curves show that the addition of 0.25% of POEMS produces bread of approximately the same tenderness as the use of 2.0% of lard.

Changes in Water Absorptive Capacity of Bread Crumb as a Measure of Staling

Farinograph Method. With the advent of the farinograph, Fuller (7) suggested that the swelling test devised by Katz (10) be modified to make use of the new instrument. Fuller preferred to titrate the dough to a definite consistency and to use the absorption thus found as an index of staling. Later Bice and Geddes (3) developed a method involving the measurement of crumb dough consistency at constant water content. Freilich (8) used their method but measured the consistency at a slightly different water content. All agreed that the farinograph offered promise as a method of measuring staling but the method did not give results comparable to those obtained by compressimetric measurements when applied to breads containing POEMS (3, 8).

Bread from hard winter wheat flour was baked by the standard procedure indicated earlier. Half the loaves contained 1% POEMS based on the weight of the flour. After being stored overnight in doubly-sealed waxed paper, the loaves were decrusted and the crumb cut into small cubes about ¼ in. to the side. The bread was stored in air-tight containers during the testing period. Fifty-gram samples of the crumb were placed in the farinograph bowl, the motor started, and water added from a pipette until a consistency of 500 Brabender units

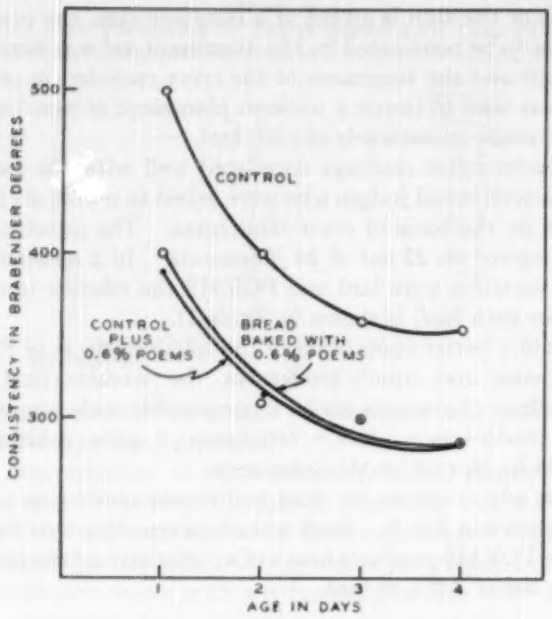

FIG. 6. The effect of age on farinograph consistencies of bread-water doughs.

was obtained for the control bread. For the control bread 20.3 ml. of water were required. Three determinations were made each day, using the same quantities of crumb and 20.3 ml. of water; one determination for the control bread, one for bread containing 1% POEMS, and one for the control bread to which the same quantity of POEMS was added in the farinograph.

The results in Fig. 6 show that for bread at any age, POEMS decreased the consistency of the crumb-dough at constant water content. POEMS added in the farinograph had the same effect as POEMS baked in the bread.

TABLE III

THE RELATIONSHIP OF POLYOXYETHYLENE MONOSTEARATE TO THE STALING OF BREAD AS MEASURED BY THE SWELLING POWER TEST

	Swelling Power: Weight of Sediment per Gram of Crumb				
Day	0% POEMS	.25% POEMS	0.5% POEMS	1.0% POEMS	2.0% POEMS
1st	3.5	3.5	3.4	3.4	3.2
2nd	3.5	3.1	2.9	2.9	2.9
3rd	3.9	3.0	3.4	3.1	2.8
4th	4.4	3.3	3.5	3.1	2.7

Swelling Power Test (Katz Method). Crumb swelling power was determined as described in Cereal Laboratory Methods, 5th Edition (5), except that the specified 25 gm. sample was reduced to 10 gm. to facilitate the subsequent determination of soluble starch in the decantate. The amount of water was proportionately reduced.

The results were most erratic (Table III). The control bread actually increased in swelling power with age while that containing POEMS remained essentially the same.

Changes in Soluble Starch Content as a Measure of Staling

According to Katz (10), most of the changes in soluble starch take place during the first eight to ten hours after the bread is removed from the oven. From a practical viewpoint this reduces the test to one of academic interest only, since no housewife receives commercial bread less than ten hours old.

TABLE IV

THE RELATIONSHIP OF POLYOXYETHYLENE MONOSTEARATE TO THE STALING OF BREAD AS DETERMINED BY SOLUBLE STARCH

Day	Per Cent Soluble Starch				
	0% POEMS	0.25% POEMS	0.5% POEMS	1.0% POEMS	2.0% POEMS
1st	4.5	4.9	4.2	4.3	4.6
2nd	4.2	4.0	4.1	4.2	4.1
3rd	3.4	4.1	3.9	3.3	4.1
4th	3.6	4.0	4.0	3.7	3.8

Bread was baked containing 0%, 0.25%, 0.5%, 1.0%, and 2.0% POEMS and soluble starch in the crumb determined at one-day intervals for four days by the method of Katz (10). The data are recorded in Table IV and are so erratic that no conclusions can be drawn regarding the effects of POEMS on the rate of staling.

Effect of POEMS on Wetting Rates of Bread Crumb

Loaves were baked containing 0%, 0.5%, 5.0% POEMS and 1.0% sodium lauryl sulfate based on the weight of flour. The sodium lauryl sulfate used was desalted before being incorporated into the dough. The breads were air-dried and ground under the same conditions to pass a 20-mesh screen.

The wetting-rate test was a modification of the one used for textiles. A weighed sample (usually 5 gm. except as otherwise indicated in Table V) of the ground bread was poured through a wide-mouthed funnel onto the surface of 450 ml. of distilled water in a 500-ml. graduated cylinder, and the time required for the material to sink was

TABLE V

RELATIVE WETTING TIMES OF DRY GROUND BREAD WITH AND WITHOUT ADJUNCTS

Adjunct	Seconds for Sample To Sink					Average
	1	2	3	4	5	
None	185	171	140.2*	176.8		177.6
5.0% POEMS	214	205	195	225		210
None**	201.2					201.2
5.0% POEMS**	696.8					696.8
0.5% sod. lauryl sulfate	55.0	62.0	72.8	51.8	45.2	51.2
1.0% sod. lauryl sulfate	30.2	40.8	55.4	32.2	49.6	41.6

* Not included in average.
** 10-gram samples.

measured with a stop watch. The instant at which the last particle of bread lost its opaque appearance was taken as the endpoint.

Table V shows that the wetting time of bread was inversely proportional to the amount of sodium lauryl sulfate added, but directly proportional to the amount of POEMS present. Bread containing no adjunct took longer to wet than bread containing sodium lauryl sulfate, but bread containing POEMS took longer to wet than the control bread. The results indicate that, unlike sodium lauryl sulfate which makes bread more hydrophilic and speeds up the wetting rate, POEMS makes bread less hydrophilic and retards the wetting rate.

Discussion

It appears that POEMS does not prevent staling; it merely retards the process. This retardation of staling may be caused by POEMS precipitating amylose which is present in a free condition in the bread. But combined with the precipitation, and probably of much more importance, is the action of POEMS on the starch granules themselves. POEMS appears to combine, probably by hydrogen bonding with alcoholic groups protruding from the starch granule, with the granules themselves. This addition of a hydrophobic moiety hinders to some extent the forces which cause alignment and hydrogen bonding of starch chains with each other and the subsequent hardness which develops when bread stales. This interference retards the staling process but does not stop it.

If this mechanism of the action of starch is acceptable, and no other mechanism appears at this time to explain the laboratory findings, it appears reasonable that those staling tests which depend on the action of water at or near room temperature cannot be expected to give normal indications. Of all the traditional tests for staling, only those involv-

ing the measurement of compressibility and crumbliness appear to give results which can be interpreted as indicating the degree of bread staling, and crumbliness appears to be a better measure of tenderness than of bread age.

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AN AUTOMATIC ELECTRICAL RECORDING PRESSUREMETER [1]

SUTTON REDFERN [2]

The pressure of the gas produced during fermentation in a sealed vessel is automatically recorded by a 12 point strip chart Brown Electronik potentiometer. Pressure is measured with a Statham Pressure Transmitter which translates pressure into a voltage linearly proportional to the pressure. The potentiometer is calibrated directly in millimeters of mercury and can record a total pressure range of 0–600 mm. Twelve separate pressures may be recorded simultaneously. The various components of the apparatus, recorder, pressure transmitters, power supply, pressure vessels, pulley box, and water bath are described.

Many papers, reviewed by Elion (6), have been published dealing with the measurement of gas production in fermenting dough. Two

[1] Manuscript received April 26, 1950. Presented at the annual meeting, May, 1948.
[2] The Fleischmann Laboratories, Standard Brands Inc., New York, N. Y.

methods are generally used to measure the amount of gas produced: the volumetric method as described by Bailey and Johnson (1) and the manometric method originated by Blish, Sandstedt, and Astleford (3), from whose work was developed the well known pressuremeter of Sandstedt and Blish (14) which is so widely used today. Both of these methods are usually manually operated, i.e., the operator takes readings every hour or even oftener. Few automatic recording types of equipment have been constructed, and of those that have been offered commerically only a very limited number are in use in this country.

Markley and Bailey (12) described a recording fermentometer in which air displaced by the expanding dough displaces water in a bell jar. This displaced water is discharged into a cylinder equipped with a float and stylus. The height of the stylus, which is proportional to the volume of displaced air, is recorded on a conventional kymograph chart.

Working and Swanson (17) constructed a simple recording pressuremeter in which the pressure of the gas produced during fermentation actuates a stylus floating in a mercury manometer. The height of the stylus, which is proportional to the pressure, is automatically recorded by electric sparks which burn holes in a chart fastened to a kymograph drum.

At least three devices for recording gas production have been offered commerically. These are the fermentograph of Brabender (2), the Chefaro balance described by Elion (5), and the S.I.A. Fermentation Recorder (9). In the Fermentograph a rubber balloon containing the fermenting dough is suspended from a recording balance in water. As the balloon expands it becomes more buoyant and the rise is recorded by the balance. Although very attractive in principle, Schmalz and Sullivan (15) showed that the error due to diffusion of carbon dioxide through the rubber balloon was too large to be neglected. The Chefaro balance is similar in principle to the Fermentograph but places the dough under an open bell jar. The S.I.A. apparatus, which is similar to the Benedict-Roth (7) spirometer, measures the volume of fermentation gas with a floating gasometer bell. As far as is known, the Fermentograph is the only one of these instruments available today.

The instruments so far mentioned can be classed as integrating instruments in that the total amount of gas is recorded. For some purposes the instantaneous rate of gas production is very important. A number of investigators (4, 16) have determined average rates by measuring the increment of gas produced during successive intervals of time where this interval has been as small as five minutes. These

data can of course be obtained from any of the integrating types of instruments.

Miller, Edgar, and Whiteside (13) constructed an instrument which automatically recorded the pressure produced during every successive ten minute interval. Landis and Frey (10) used a sensitive optical type differential recording manometer to measure the instantaneous rate of fermentation. Earlier, James and Huber (8) made similar instantaneous measurements by allowing the water displaced from a tank by the fermentation gas to flow through an orifice under gravity. The height of water above the orifice was proportional, although not linearly, to the rate of gas production and the height was recorded by a stylus floating in the water.

The development during recent years of improved recording potentiometers, electrical pressure transmitters, and power supplies have made it possible to construct the automatic electrical recording pressuremeter described in this paper. This recording pressuremeter can be assembled from readily available standardized components. Such an instrument should make it possible to examine more exactly the entire course of yeast fermentation in dough.

Materials and Methods

General. A general view of all components except the power supply is shown in Fig. 1. The pressure of the gas produced during fermentation by a piece of dough in special sealed cups is automatically recorded by the 12-point strip chart Brown Electronik potentiometer shown on the left. The potentiometer is calibrated directly in millimeters of mercury and can record a total pressure range of 0 to 600 mm. Pres-

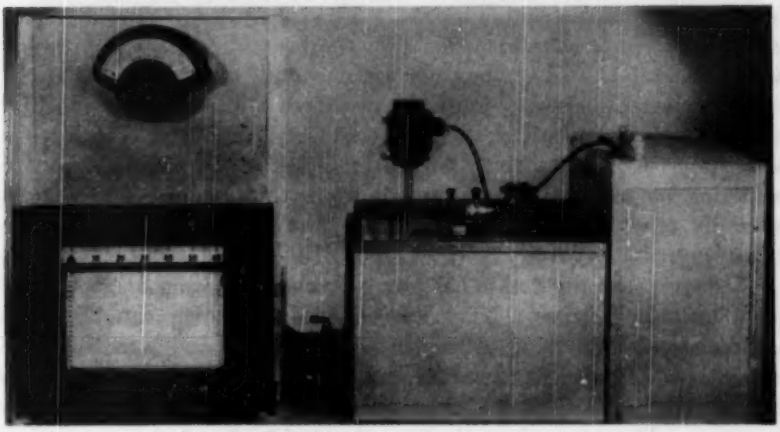

FIG. 1. General view of recording pressuremeter.

sure is measured with a Statham pressure transmitter which converts pressure into a voltage linearly proportional to the pressure. A pressure transmitter mounted on its cup in place in the water bath is shown in the center of Fig. 1. On the right is the so-called pulley box. This contains the necessary wiring for connecting each pressure transmitter to the potentiometer and to the power supply and the zero adjustment and calibrating resistors. The pulley box is connected to the potentiometer with a 30-wire cable. The voltmeter mounted over the potentiometer indicates the voltage of the power supply.

Statham Pressure Transmitter. The heart of the pressuremeter is the Statham pressure transmitter shown mounted on a pressure cup in Fig. 2. The pressure to be measured is applied through the bottom opening to a relatively slack metal bellows and the resulting displacement of the bellows is measured with a Statham unbonded resistance wire strain gage transducer element. Of the various models of pressure transmitters available, Model P6-12D-250 was chosen as most suitable. This transmitter is designed to measure 12 lb. maximum pressure and has a nominal electrical resistance of 250 Ω. The output is approximately 36 mv. with an input voltage of 12 v.

Pressure Cup. Special pressure cups, $2\frac{1}{2}'' \times 4''/_{16}''$, approximately 300 cc. in volume, were constructed of stainless steel. This cup, shown in Fig. 2, is patterned after the small De Vilbiss paint spray cup used by Malloch (12). The cup body is made from $2\frac{1}{2}''$, 16 gauge (approximately 1/16″), stainless steel sanitary tubing. This tubing comes already polished so that it needs no further finishing. The bottom and the ring at the top were induction soldered to the tubing with silver solder. The extra ring at the top was added to give increased gasketing surface. The cover is turned from solid bar stock in the form of a frustrum of a cone. A 3/32″ thick soft rubber gasket is cemented in the annular space on the bottom of the cover. The screw is made from $1\frac{1}{8}''$ hexagon stock. The stem, turned from 1″ round bar stock, has a $\frac{1}{8}''$ tapered pipe thread at the top for connection to the pressure transmitter and a 7/16-20 thread at the bottom which is soft soldered to the cover. In the future it is planned to eliminate soldering by using $\frac{1}{8}''$ pipe threads for both threads. The yoke is specially cast from phosphor bronze. The release valve is a Hoke blunt point baby valve.

This pressure cup design has proved very satisfactory. No trouble from leaks or corrosion has occurred, and the cover can be easily tightened without using a special holding stand and wrench. The entire assembly may be tested for leaks by attaching a hose connection to the valve, adding 10 to 12 lb. air pressure, and immersing the whole in water. The most minute leak can be readily detected.

Recording Potentiometer. The output from each pressure transmitter is recorded on a Brown Electronik strip chart potentiometer (series 153 X 65, fast speed, synchro-printing, 12 record). This model prints 12 multi-colored records by means of a print wheel. Each record consists of one of six different colored numbers and plus signs, each plus sign (+) representing the exact pressure and time coordinate. Chart speeds of 1, 2, 3, and 4 inches per hour are obtainable with the change

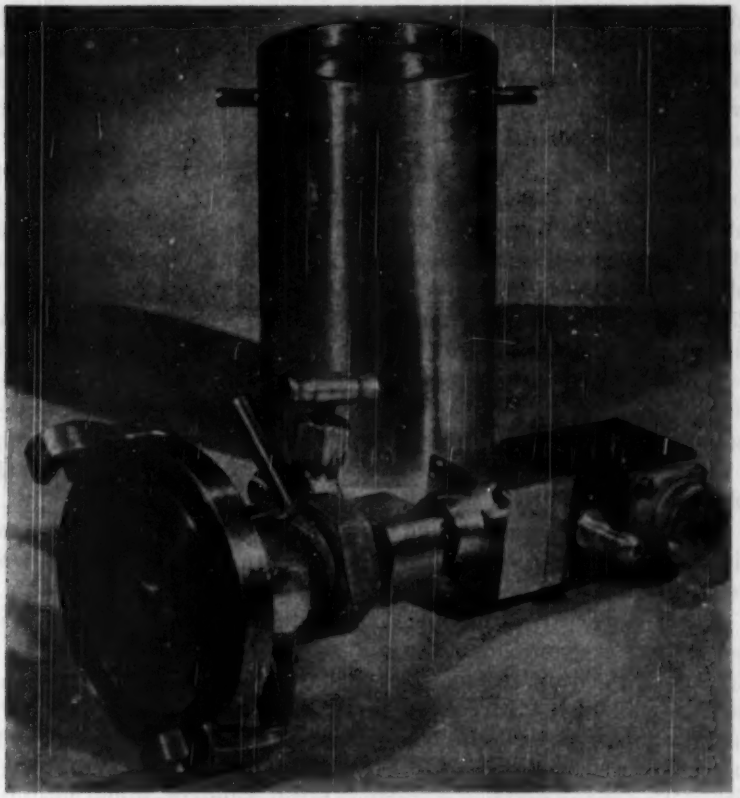

FIG. 2. Pressure cup and Statham Pressure Transmitter.

gears furnished, although 4″ per hour is used for fermentation times lasting 4 to 6 hours. The scale has a calibrated length of 11″ and is evenly graduated 0 to 60 equal to 0 to 600 mm. of mercury pressure.

The actual electrical span is 25 mv., which was deliberately chosen smaller than the maximum output available from the pressure transmitters. This makes it possible to use transmitters of varying sensitivity as it is very difficult for the manufacturer to exactly predeter-

mine the output of individual pressure transmitters. Matching of the transmitter output to the recorder is described under the heading of calibration.

It was found necessary to connect a one microfarad paper capacitor from the positive terminal in the recorder to ground in order to by-pass a stray alternating current component picked up by the pressure transmitters or their connecting wires from the Nobatron power supply.

The recording potentiometer contains an integral commutator switch which connects each pressure transmitter in sequence. The connections from the switch terminal block in the instrument are all brought to terminal strips placed below the voltmeter, and which in turn are connected to a Type K, 34-wire Cannon connector mounted on the back of the potentiometer mounting frame.

Power Supply. A Model E-12-5 Nobatron made by the Sorensen Company, is used to supply constant voltage direct current for the input to each pressure transmitter. The Nobatron which is electronically controlled will supply 5 amp. at 12 v. with a variation in output voltage of less than 0.5%, even when the line voltage varies from 95 to 125 v., and the load varies from 2.5 to 5 amp. Since 12 pressure transmitters will require not more than 1 amp., a 5 Ω., 50 w., bleeder resistor is shunted permanently across the output of the Nobatron to insure a minimum load of 2.5 amp. and bring the load within the range of best regulation. The output voltage may be varied over a limited range of 12 ± 10% v. but is maintained constant at 12 v. as measured by the voltmeter mounted over the potentiometer (Fig. 1).

The Nobatron was mounted as far away from the pressure transmitters as possible in order to minimize pickup of stray currents from the strong magnetic fields surrounding it.

Pulley Box. All wiring and controls for operation of the pressure transmitters are consolidated in the pulley box shown on the right in Fig. 1. The wiring although simple in principle is complex in that there is a lot of it. Moreover, some ready means must be used to quickly connect the pressure transmitters at varying distances from the pulley box without permitting the connectors to fall into the water bath. This entire problem was solved by modeling the pulley box after a telephone switchboard.

The wiring connections necessary for each pressure transmitter are shown diagrammatically in Fig. 3. Terminals 1, 2, 3, and 4, are numbered to correspond to the terminals of the Wheatstone bridge circuit in the pressure transmitter. Terminals 2 and 3 are connected permanently to terminal strips. Terminals 4 and 5 are connected to bus bars which in turn are connected respectively to the positive and negative sides of the power supply. Resistor R_3 is a 200 Ω wire wound

rheostat used for calibration of each pressure transmitter. The zero point is adjusted with resistor R_1 which has a total resistance of 30,000 Ω and consists of a 10,000 Ω wire-wound potentiometer in series with two 10,000 Ω fixed resistors, one on each side. Resistor R_2 is 15,000 Ω. S is a single-pole, single-throw toggle switch used to short terminals 2 and 3. Without this switch the recorder would be connected to an open circuit when the pressure transmitter is not in use. Under this condition the recorder drifts and may print any place on the chart. The shorting switch is, of course, opened when the pressure transmitter is connected. Each pair of output wires and the two wires to the voltmeter are cabled together and soldered permanently to a 34-wire

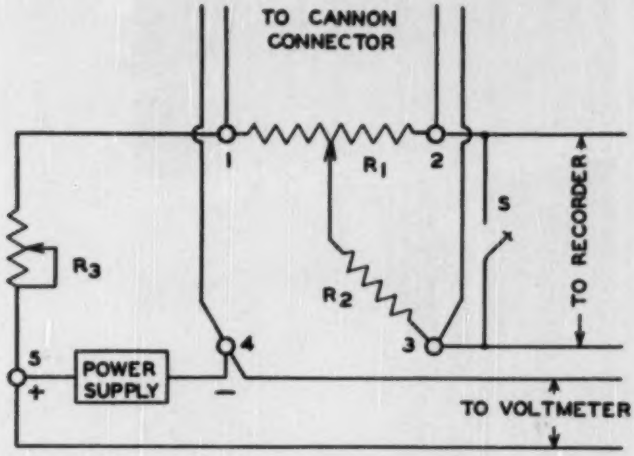

FIG. 3. Electrical wiring diagram used in pulley box for each pressure transmitter.

Cannon connector mounted on the side of the pulley box. The pulley box is connected to the recorder with a 30-wire cable and Cannon connectors. This allows the pulley box and recorder to be permanently wired and still be at any distance from each other.

Referring now to Fig. 4 which shows the interior of the pulley box, the type K Cannon connectors which connect to corresponding mating connectors on the pressure transmitters are on the top. The connectors lock together with a screw collar. The 4-wire, 16 gauge, rubber-covered cable attached to each connector is clamped at the other end, and hangs in a loop from the center of which is suspended a 1 lb. lead weight attached to a freely movable pulley. The cable loop is long enough so that the cable may be extended about 40″ from the box. There is very little chance of dropping the connector in the water

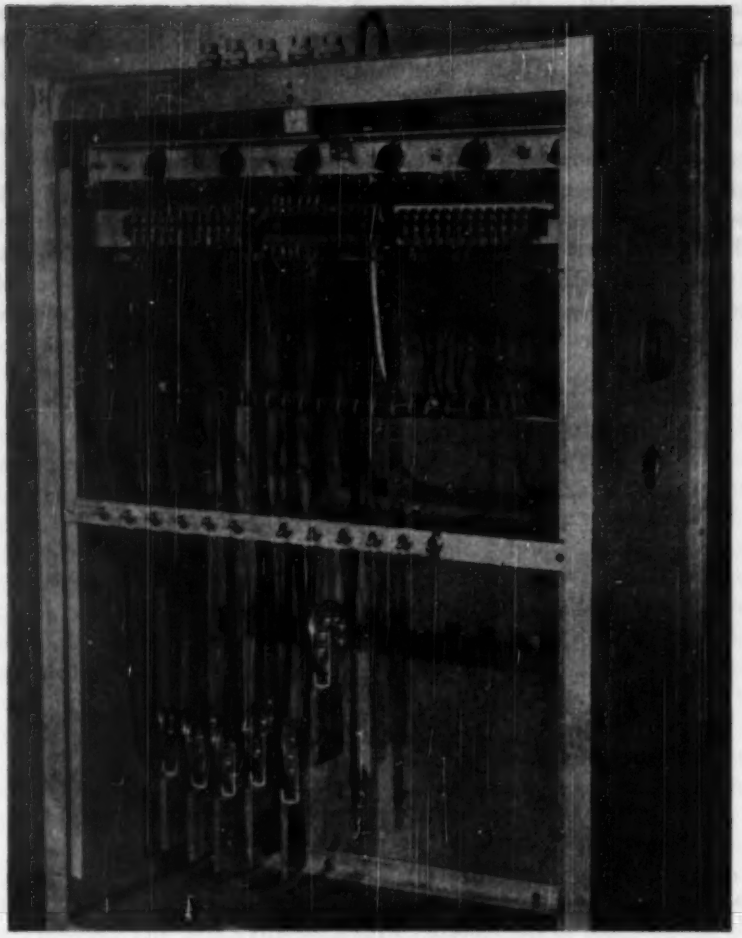

FIG. 4. Interior view of pulley box.

bath as the weighted pulley draws it back into the box whenever it is released.

The terminal strips, bus bars, zero adjustment, calibrating resistors, and shorting switches are readily identified in Fig. 4. The shaft of the calibrating resistor is slotted which allows it to be adjusted only with a screwdriver which prevents it from being accidentally turned.

The entire wiring arrangement proved to be very flexible since a number of variations and additions were made directly to the terminal strips without disturbing existing wires. It is planned eventually to mount each shorting switch conveniently adjacent to its respective Cannon connector on the top of the pulley box.

Water Bath. The pressuremeters are immersed in a conventional thermostatically controlled water bath (Fig. 1) containing a 12 place rack. The holes in the rack are diagonally arranged so that the wires to the pulley box are separated.

Calibration of Pressure Transmitters. Although the pressure transmitters are factory calibrated, the output of different transmitters cannot be made to give exactly the same output voltage for the same pressure. The input voltage must be adjusted until the pressure indicated on the potentiometer equals the pressure measured with a mercury manometer. This is done by attaching each pressure transmitter and cover to the calibration unit shown in Fig. 5 made from a Sandstedt-

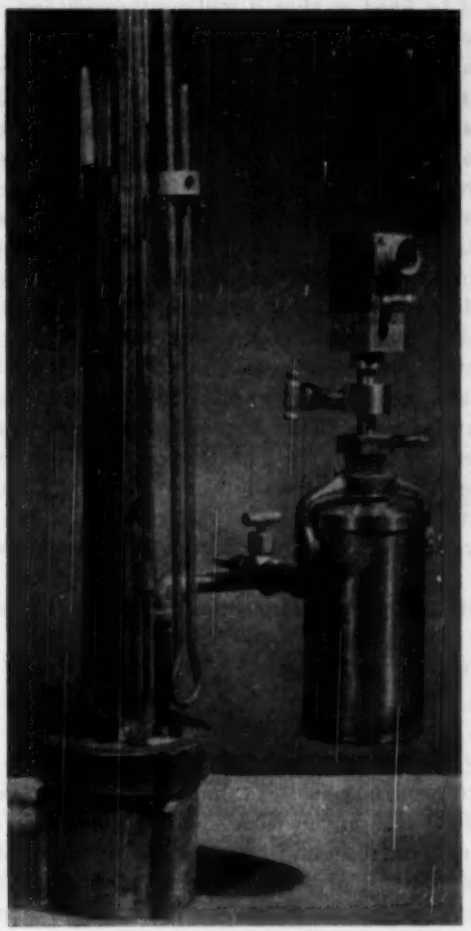

FIG. 5. Apparatus used for calibrating pressure transmitters.

Blish manometer pressuremeter. Air pressure is applied through the Hoke valve and the calibrating resistor adjusted until the pressure read on the potentiometer equals the pressure read on the manometer. A calibrating pressure nearly equal to full scale is used and the calibration is repeated until one is certain that the calibration is correct.

Operation of Pressuremeter. The sequence of operations in the use of the pressuremeter is as follows: The Nobatron is turned on 10 to 15 minutes before use in order to let it warm up and develop a stable output and the output is adjusted to 12 v. The Brown Electronik potentiometer should be operated according to the directions of the manufacturer. The power supply is best left on continuously since this procedure is stated to give longer tube life. The wiring of the potentiometer should be fixed so that it will continously indicate even when the chart drive switch is turned off.

After each pressure transmitter is connected to the pulley box, it requires about a five minute warm-up period before the output becomes steady. This is particularly noticed with the zero setting and one should not be in haste to make any changes in the zero adjustment until the warm-up period is over. When five minutes or more has elapsed, the output with no pressure applied to the transmitter is set to zero with the adjusting resistor. There are slight daily zero shifts of about ± 2 mm., and for the highest accuracy the zero should be adjusted each day. The amount that the resistor must be moved is readily correlated with the amount of zero shift.

To measure fermentation pressure, a suitable sized dough aliquot, which may vary from 25 to 50 gm. is placed in the cup, or alternately the dough may be mixed in the cup and the cover affixed tightly with the valve open. The cup is then immersed in the water bath, the pressure transmitter connected to its corresponding Cannon connector, and the shorting switch opened. After at least five minutes have elapsed, the zero point is adjusted and the valve closed exactly ten minutes from start of mixing.

The potentiometer chart is positioned so that the first point prints on one of the principal time lines. Successive doughs can be started as close as five minute intervals.

Results

Relation of Response to Pressure. According to the manufacturer, the pressure transmitters exhibit a linear relationship between applied pressure and electrical output with an error of less than one per cent of full range from hysteresis and creep. The linearity of response was tested by attaching one of the pressure transmitters to the calibration unit and comparing pressures measured on the manometer with the

TABLE I

RESPONSE OF PRESSURE TRANSMITTER

Pressure	
Potentiometer mm.	*Manometer* mm.
0	0.0
51	51.5
100	101.0
150	151.0
200	201.5
250	251.5
300	301.0
350	351.5
400	400.0
450	449.0
500	499.0
550	548.0
599	597.0

pressures indicated on the potentiometer. The data, shown in Table I deviate only slightly from a linear relationship. In general, the performance of the pressure transmitters has been better than the stated 1%, probably because they are used essentially for measurement of static pressures. The pressure changes are relatively slow and each transducer element is essentially always in a state of equilibrium, which reduces any hysteresis or creep effects.

The pressuremeter may be used for any method for which the manually operated pressuremeters have been used. Typical curves obtained for doughs containing 3% yeast, no salt, and 0, 1, 2, 3, and 5% sugar are shown in Fig. 6. The transference from sucrose fermen-

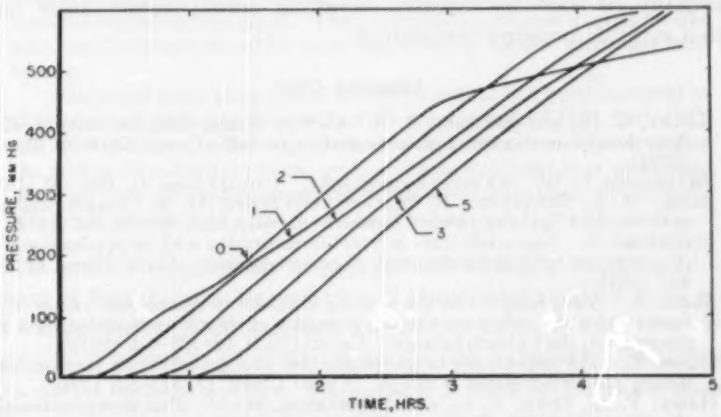

FIG. 6. Typical pressuremeter curves obtained for doughs containing 3% yeast, no salt, and 0, 1, 2, 3, and 5% sucrose. (In the chart actually obtained from the recorder, the time axis runs from right to left.)

tation to maltose fermentation is clearly shown by the inflection points in the curves from doughs containing none, 1 and 2% sugar. With 5% sugar, the inflection point has disappeared. Differentiating the pressure time curves would show this condition more emphatically, but it is believed that these are the first curves which show this transition so clearly.

Some idea of the replicability of the recording pressuremeter is shown by Table II. These are the hourly rates and total pressure of six aliquots of the same dough. Zero time is taken as ¾ hour after mixing. The reason for choosing this starting time is because although all the cups were placed in the water baths simultaneously, recording was started at successive five minute intervals in order to separate the curves. The initial portion of most of the curves is thereby missed.

TABLE II

REPLICABILITY OF PRESSUREMETER READINGS
(Six 25 gm. aliquots from same dough)

Hour	*Pressure* *Range* *mm.*	*Average* *mm.*
1	86–88	87
2	115–117	116
3	118–120	119
4	108–110	109
5	99–100	100
Total	527–532	530

This new recording pressuremeter has not been in operation long enough to have explored its many uses and possibilities. It may eventually be used for directly recording actual fermentation rates with a suitable pressure transmitter.

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WET MILLING OF GRAIN SORGHUM [1,2]

R. L. ZIPF, R. A. ANDERSON, and R. L. SLOTTER [3]

ABSTRACT

Studies on the recovery of starch, gluten, and oil from grain sorghum showed that this grain could be processed satisfactorily by methods similar to those employed industrially for the wet milling of corn. Optimum separations of starch and gluten were obtained when grain sorghum was steeped at 110° to 120°F. for 24 hours or longer, in a solution of 0.2 to 0.25% sulfur dioxide in water. To produce sorghum starch of comparatively low protein content (0.35 to 0.7%) it was necessary to increase the pitch of the starch table over that used for wet milling corn.

Some varieties of grain sorghums have a highly pigmented layer. When this layer was removed by pearling, a whiter starch could be produced than from the whole sorghum grain. The hull fraction contained a carnauba-like wax, small amounts of which were recovered by solvent extraction with hexane.

Samples of grain sorghums of two varieties that had been harvested at three moisture levels and dried with air at temperatures of 125°, 150°, 175°, and 200°F. were wet milled to determine the effect of such artificial drying on the milling characteristics of the grain. It was concluded that no damage detrimental to wet-milling operations occurs when grain sorghum is artificially dried, provided the grain is not dried to less than 11 to 13% moisture.

The grain sorghums are important economically to the semi-arid regions of this country since they will thrive and produce both grain and forage under conditions that cause other crops to fail. In recent years the production of sorghum grains has increased tremendously

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[2] Contribution of Northern Regional Research Laboratory, Peoria, Illinois. One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.
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TABLE I

CONSTITUENTS OF CORN AND GRAIN SORGHUM

	Corn	*Sorghum*
Starch % d.b.	70.6	69.5
Protein % d.b.	10.2	13.0
Oil % d.b.	4.3	3.6
Ash % d.b.	1.6	1.9
Germ % d.b.	11.1	10.5
Bran % d.b.	6.5	6.3

and industrial utilization of the crop has been encouraged by the development of varieties which can be harvested with combines. Before 1943 (5) almost all of the sorghum grain and forage produced was consumed on the farm. Only a small quantity of the grain was used for food or in industry. During the war, the shortage of corn forced producers of alcohol and starch to utilize grain sorghums. It was found that the grain could be processed much like corn. Since the end of war, a large plant has been erected in Texas to wet mill grain sorghums for the production of starch, glucose syrup, and dextrose (6).

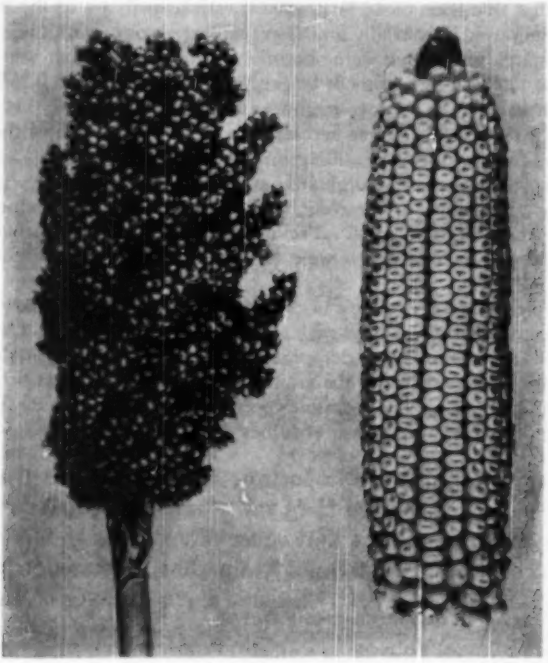

FIG. 1. Grain bearing structures of corn and grain sorghums.

Grain sorghum is a comparatively new industrial crop and a standard procedure for wet milling the grain has not been established. The industrial facilities for the wet milling of corn are extensive, however, and considerable information on the methods employed has been accumulated (2). Hence, it was logical that a procedure for wet milling grain sorghum be an adaptation of that used for corn. As shown in Table I, grain sorghum is similar in composition to corn, although in appearance it is quite different (Fig. 1). The present study was conducted to determine the suitability of the standard wet milling process for use with grain sorghum, and to ascertain the effect of variations in processing conditions on the quality and yield of products obtained from grain sorghum.

A subsequent investigation was conducted in which samples of artificially dried grain sorghums were wet milled to determine the effect of such drying on the milling characteristics of the grains. The work was conducted on samples of dried grains prepared and supplied by the Corn Products Refining Company, and the Texas Agricultural Experiment Station, College City, Texas. The results obtained by both the Texas group and the Northern Laboratory have been published (10) but a brief account of the wet-milling experiments is included in this report.

Procedure

The general process utilized in the laboratory for the wet milling of grain sorghums is shown in Fig. 2. The procedure is approximately the same as the industrial process for the wet milling of corn, and with modifications it can be used to wet mill wheat (9).

A 4-lb. sample of grain is steeped with 2,800 ml. of distilled water containing sulfur dioxide. The operation is conducted in the equipment shown in Fig. 3. Grain is charged into the insulated bottle and covered with the dilute sulfurous acid which is prepared by bubbling gaseous sulfur dioxide into distilled water. The steeping agent is pumped continuously from the bottle through a screened opening to an oil bath from which it is returned to the bottle. The oil bath is equipped with a 1,500 watt heater and thermostat, and serves to maintain the circulating liquor at a constant temperature during steeping. Industrial steeping of corn is conducted in a countercurrent multiple contact system for about 42 hours. An important difference between the two methods is that little if any lactic acid is formed during the laboratory steeping. However, laboratory experiments with corn have have shown that starch yields comparable to those of industry are obtained by steeping the grain batchwise for 24 hours.

After the grain is steeped, the water is drained off, measured, and analyzed. The steeped grain is ground in a Quaker City drug mill,

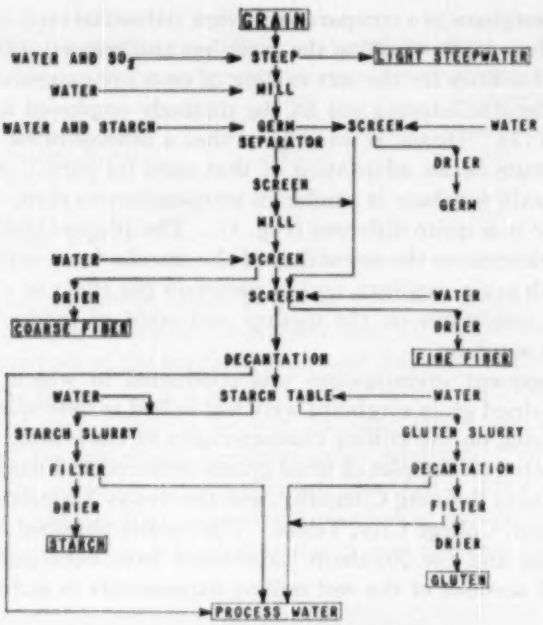

FIG. 2. Flowsheet for the wet milling of grain.

Fig. 4, to free the germ and hull from the rest of the kernel. To facilitate floating the germs from the 4 lb. of ground grain, 200 gm. of starch is added to the slurry, the specific gravity of which is adjusted with additional water to 7 to 7.5° Bé. at a temperature of 80° F. The germ material, containing a small proportion of crushed germs, is washed by hand on a 0.039-in. perforated copper screen measuring about 14 in.

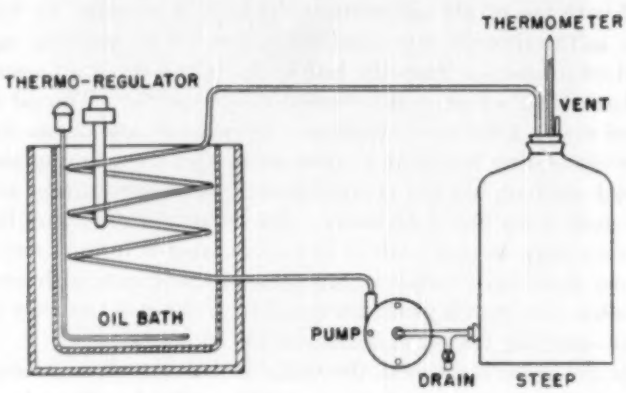

FIG. 3. Laboratory equipment for the steeping of grain sorghum.

square. The germs and 1 l. of water are mixed before each of three washes.

The slurry from which the germ has been removed is screened and the material retained on the sieve is ground again with a closer setting on the mill in order to free more starch from gritty or unbroken particles. Coarse fiber is removed from the ground mass by screening it through a 0.039-in. perforated copper sieve. The coarse fibers are washed with three 1-l. portions of water. The screened slurry is combined with the germ washings and fiber washings and the resultant liquor is screened on a 200-mesh stainless steel sieve, to remove the

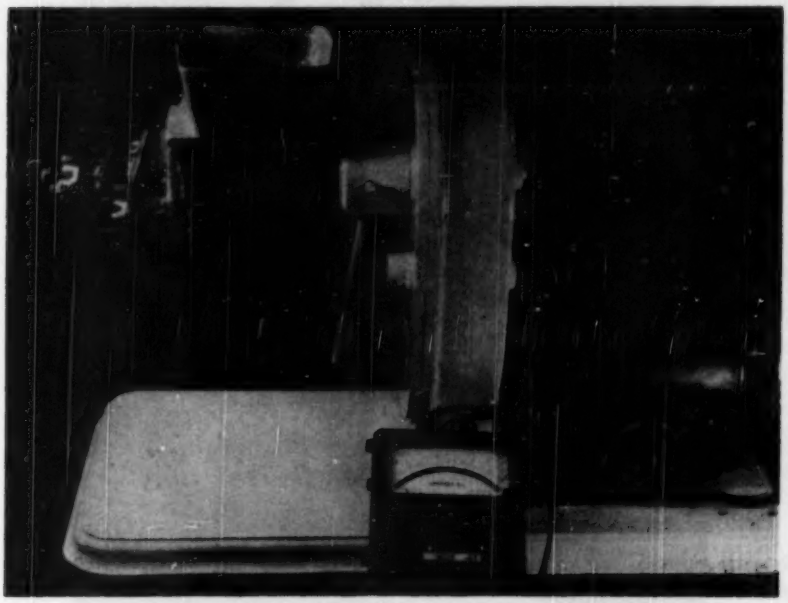

FIG. 4. Mill in which steeped grain is ground.

fine fiber, which is then washed. The final slurry containing the starch and gluten is settled for about 2 hours and excess water is decanted. The specific gravity of the liquor is adjusted to 6° Bé. at 100°F. before it is passed to the starch table. The head of the table, the mill-starch tank with accessories, and a constant-head device are shown in Fig. 5. This table, the pitch of which may be varied, is 4 in. wide and 20 ft. long. The starch that settles on the table is resuspended in water and filtered, using a Buechner funnel. The gluten is recovered from the table overflow by allowing it to settle overnight, syphoning off the supernatant water, and then filtering the settled gluten. All decanted

water and filtrates are combined, measured, and analyzed. The germs, coarse fiber, fine fiber, starch, and gluten are put on separate stainless steel trays and dried overnight at a temperature of 120°F. in a Proctor and Schwartz shelf oven.

Analytical Methods. Kjedahl nitrogen in the various products was determined by the Kjeldahl-Gunning-Arnold method (1). Protein was calculated by means of the formula N × 6.25. Starch was determined polarimetrically by the procedure of Earle and Milner (4). The

FIG. 5. Laboratory starch table and appurtenances.

viscosity of starch was measured according to the Scott method for hot paste viscosity (7) which is a special test used by the wet millers to evaluate their product. To determine moisture, samples were dried for 4 hours at 110°C. under a vacuum of 28 in. of mercury. The sulfur dioxide content of the steep water was ascertained by titration with a potassium iodide-iodine solution using starch as an indicator. In this paper concentration of sulfur dioxide is reported as per cent, equivalent to grams of gas per 100 ml. of solution.

Experiments and Results

The Effect of Concentration of Sulfur Dioxide in Steepwater. In this and subsequent experiments the same sample of Martin Variety of grain sorghum was used unless otherwise noted. Several experiments were conducted to determine the effect of concentration of sulfur dioxide in steepwater on the wet milling of grain sorghum. It is known that excessive concentrations of sulfur dioxide have a detrimental effect on cornstarch (3), and cause drastic changes in root starches. Insufficient sulfur dioxide in steepwater usually results in lower starch yields. Only the sulfur dioxide content of the steepwater was varied in these experiments. In all runs the grain was steeped for 65 hours at 118°F. The data procured in these tests are given in Table II.

When the steepwater contained less than 0.20% sulfur dioxide, poor germ separation was obtained. Very few germs floated and the fiber content of the germ fraction was high. Germ separation was satisfactory with 0.20% and 0.25% sulfur dioxide in the steepwater.

TABLE II

EFFECT OF CONCENTRATION OF SULFUR DIOXIDE IN STEEPWATER ON WET MILLING OF GRAIN SORGHUM

SO_2 in Steepwater[1]	Starch Recovered, d.b.	Protein in Starch, d.b.	Protein in Gluten, d.b.	Starch in Gluten, d.b.	Protein in Steepwater Solids	Viscosity of Starch, Scott
%	%	%	%	%	%	*sec.*
0.10	74.2	0.89	39.4	45.3	1.61	127
0.15	71.4	0.73	40.3	46.2	1.64	117
0.20	74.1	0.98	37.9	53.0	1.77	113
0.25	75.5	0.62	40.5	47.9	1.80	118

[1] % = gm./100 ml.

Varying the sulfur dioxide content of steepwater between 0.10 and 0.25% had no significant effect on the starch recovery, the protein content of the recovered starch, protein and starch contents of the gluten, or the viscosity of the starch. A slight increase in the protein content of the steepwater was obtained at the higher concentrations of sulfur dioxide. Since no detrimental effects were caused by 0.25% sulfur dioxide, all subsequent work was conducted with steepwater containing this quantity of the gas.

Temperature of Steeping. All samples of grain were wet milled in the regular manner but steeping temperatures of 110°, 120°, 130°, 140°, and 150°F. were used. In all cases the grain was steeped for 65 hours with water which contained 0.25% sulfur dioxide. The results of the experiments are given in Table III.

The temperature of steeping had an outstanding effect on the tabling operation. As the temperature was increased, the starch recovery, the viscosity of the starch, and the protein content of the gluten were reduced, and the starch content of the gluten increased. The quality of both the starch and gluten were impaired drastically at the high steeping temperatures. However, microscopic examination of the starch revealed that gelatinization as indicated by loss of birefringence of the granules, had not occurred at any of the temperatures. This was as expected since sorghum starch begins to gelatinize at about 158°F.

Since the starch was not damaged appreciably when the grain was steeped at 120°F., it was concluded that grain sorghum should be steeped at a temperature of 110°–120°F. with water containing 0.25% sulfur dioxide.

TABLE III

EFFECT OF STEEPING TEMPERATURE ON THE WET MILLING OF GRAIN SORGHUM

Steep Temp.	Starch Recovered, d.b.	Protein in Starch, d.b.	Protein in Gluten, d.b.	Starch in Gluten, d.b.	Protein in Steepwater Solids	Viscosity of Starch, Scott
°F.	%	%	%	%	%	sec.
110	78.4	0.78	49.6	33.8	1.78	115
120	72.4	0.77	37.6	51.0	1.86	105
130	69.6	0.86	41.3	46.4	1.58	86
140	59.0	0.61	34.2	55.8	1.31	71
150	59.0	0.79	24.5	67.2	1.30	55

Time of Steeping. In the wet milling of corn on a laboratory scale, it had been determined that a steeping period of 24 hours was adequate when the operation was conducted batchwise. Several lots of grain sorghum were steeped for 24 and 71 hours and the grain then milled. It was found that the quality and recovery of starch, gluten, and germs were not improved by the longer period of steeping. As with corn, batchwise steeping for 24 hours in the laboratory method is satisfactory for the wet milling of grain sorghum.

Pitch of Starch Table. In the previous experiments sorghum mill starch was tabled under conditions which had been found to be satisfactory for the separation of cornstarch and gluten. The starch table was inclined at a pitch of ⅞ in. per 20 ft. of length which is the pitch used industrially by several processors of corn. When corn was wet milled in the laboratory equipment with the table set at this pitch, a good recovery of starch was obtained and the protein content of the starch was low, 0.30 to 0.40% (d.b.). In the experiments with grain sorghum it was noted that tabling was poor in some cases; the settled starch contained numerous pockets of gluten and the protein content

of the recovered starch was high, 0.7–1.0% (d.b.). In an attempt to improve the quality of the sorghum starch a few exploratory runs were made in which the sorghum mill starch was tabled with the unit set at a pitch of 1¾ in. per 20 ft. The results were encouraging and a series of experiments was conducted to investigate the effect of this variable.

The grain was steeped for 24 hours at a temperature of 118°F. in water containing 0.25% sulfur dioxide. In order to expedite the tests the recovery of germs by flotation was eliminated. The steeped grain was ground twice and germs and fibers removed from the slurry by the regular screening operations.

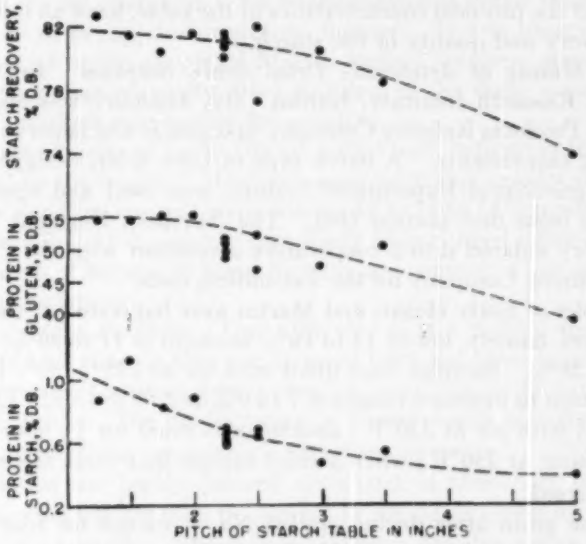

FIG. 6. The effect of table pitch on laboratory separation of starch, and protein in wet milled grain sorghum.

In the experiments the slope of the starch table was varied from 1¼ in. to 5 in. per 20 ft. of table length. The results of the tests are shown graphically in Fig. 6. The correlation between pitch of table and starch recovery, protein in starch, and protein in gluten, is not precise, but a very definite trend is indicated. As the pitch of the table was increased, the protein contents of the starch and gluten, and the starch recovery decreased. The best starch contained 0.39% protein (d.b.) and was obtained when the table pitch was 5 in. per 20 ft. However, only 73.7% of the starch in the grain was recovered, and the comparatively large quantity of starch in the overflow from the table reduced the protein content of the gluten.

Based on the results of the tests it was concluded that for the wet milling of Martin sorghum, the starch table should be pitched at 2¼–2½ in. per 20 ft. of table length. Under these conditions the starch recovery was good and the protein content of the recovered starch, 0.6–0.7% (d.b.) was improved over results obtained in previous experiments.

It should be understood that optimum pitch of a starch table is not simply a function of the grain to be processed. The effect of the grain, or rather of the characteristics of the starch and gluten that it contains, is most important. However, other factors such as steepingconditions, milling of the grain, the rate at which mill starch is pumped to the table, and the physical characteristics of the table, have an influence on the recovery and quality of the starch.

Wet Milling of Artificially Dried Grain Sorghum. In 1947, the Midwest Research Institute, Kansas City, Missouri, was engaged by the Corn Products Refining Company to organize and supervise a series of drying experiments. A batch type of farm drier, designed by the Texas Agricultural Experiment Station, was used and operated by engineers from that station (10). The Northern Regional Research Laboratory entered into a cooperative agreement with the Corn Products Refining Company for the wet-milling tests.

Samples of Early Hegari and Martin were harvested at three moisture ranges, namely, low or 14 to 16%, medium or 17 to 20%, and high or 21 to 26%. Samples were dried with air at 125°, 150°, 175°, and 200°F. down to moisture ranges of 7 to 9% and 11 to 13%. One batch was dried with air at 230°F.; another was dried for ½ hour at 150°, then ½ hour at 230°F.; while a third sample had these second conditions reversed.

All the grain after drying graded No. 1, except for four samples which had a very slight toasted odor. The moisture content of the samples ranged between 10.1 and 12.3% at the time the samples were wet milled.

Four-pound samples of each batch of artificially dried grain were wet milled under identical operating conditions. Duplicates were run for each sample in the series. The grain was steeped for 24 hours at 118° to 120°F. with 2,800 cc. of a solution consisting of 0.20 gm. sulfur dioxide per 100 cc. of distilled water.

The steeped sorghum was milled according to the simplified procedure mentioned previously.

The specific gravity of the mill starch was adjusted to 6° Baumé (60°F), and the mixture then tabled at room temperature for the separation of starch and gluten. The table used was a painted 4-in.

channel iron, 20 ft. long, with a pitch of 2½ in. for its entire length. A nozzle was used to feed the mill liquor to the table at a rate of 295 cc. per minute.

The results of the investigation are given in Table IV.

In most of the tests with Martin variety sorghum, the grain milled well. The data shown in Table IV indicate that some damage was sustained by the samples of medium and low moisture content that were artificially dried to 7 to 9% moisture. This was revealed by the lower starch yields and increases in the protein content of the starch. All of the samples harvested with a high moisture content, processed and tabled very well. Evidently these samples were not damaged during the drying operation. No relationship was noted between the temperature of drying and apparent damage. It was observed that the samples of artificially-dried Martin variety sorghum were harder after steeping than naturally dried sorghum of the same variety and, therefore, required more power for grinding.

All of the samples of Early Hegari milled well, indicating they were not injured by the drying. However, samples of this variety harvested with a low moisture content were dried only to 11 to 13% moisture and not to 7 to 9%. It is possible that Early Hegari may suffer damage, as did Martin, if harvested when its moisture content is low and dried to 7 to 9% moisture.

Pearled Grain Sorghum. One difficulty in wet milling some varieties of grain sorghum is that the pigment from the outer layers of the kernel permeates the grain during steeping, resulting in the production of colored starch. The product may be tinted shades of yellow, pink, or purple, depending upon which variety is processed. Although in some varieties the highly colored undercoat is absent at maturity, enough coloring matter may be present in the outer layers of the bran to tint the final starch. The glumes also are a possible source of color, but most of these are removed when the grain is cleaned. Sorghum breeders are striving to perfect varieties for industrial use that are free from coloring matter. Fortunately, the combine varieties, Martin and Early Hegari, give starches that are not too highly colored.

It was thought that, if the coloring layers of the sorghum were removed prior to steeping, a whiter starch could be made. With this in mind, tests were conducted to compare the wet milling of whole and pearled sorghum. Pearling was accomplished by passing the grain through a laboratory Strong-Scott barley pearler in which 100 gm. portions of grain were retained for 10 to 15 seconds. This resulted in the removal of 15 to 20% of the weight of the grain as bran and material which passed through a 12-mesh screen.

TABLE IV

WET MILLING OF ARTIFICIALLY DRIED GRAIN SORGHUMS

Martin Dried to 11–13% Moisture

	Original Moisture % 14 to 16				Original Moisture % 17 to 20						Original Moisture % 21 to 26				
Drying Temperature °F.	125	150	175	200	125	150	175	200	150 230	230 150	125	150	175	200	230
Starch Yield % d.b.	83.0	82.2	83.7	84.1	82.0	85.0	82.5	82.9	84.2	84.4	80.5	82.6	85.7	88.0	82.0
Protein in Starch % d.b.	0.33	0.33	0.32	0.33	0.36	0.34	0.33	0.34	0.34	0.33	0.32	0.38	0.36	0.36	0.31
Protein in Gluten % d.b.	38.0	36.3	37.2	39.2	43.5	39.5	38.9	37.8	41.2	38.3	45.3	44.6	40.6	41.4	36.8

Martin Dried to 7–9% Moisture

	Original Moisture % 14 to 16				Original Moisture % 17 to 20				Original Moisture % 21 to 26			
Drying Temperature °F.	125	150	195	200	125	150	175	200	125	150	175	200
Starch Yield % d.b.	77.2	78.7	77.0	79.6	81.4	79.8	79.3	82.2	83.4	83.4	83.2	82.7
Protein in Starch % d.b.	0.51	0.53	0.72	0.60	0.37	0.40	0.41	0.34	0.30	0.36	0.35	0.37
Protein in Gluten % d.b.	39.5	40.1	37.0	38.9	32.9	37.5	38.0	33.2	36.7	40.6	37.3	37.4

Early Hegari Dried to 11–13% Moisture

	Original Moisture % 14 to 16				Original Moisture % 17 to 20			
Drying Temperature °F.	125	150	175	200	125	150	175	200
Starch Yield % d.b.	83.6	84.4	83.2	84.8	83.7	84.3	83.4	81.4
Protein in Starch % d.b.	0.36	0.43	0.41	0.42	0.39	0.36	0.38	0.39
Protein in Gluten % d.b.	37.2	38.1	36.9	37.7	38.1	36.6	36.9	35.0

To 7–9% Moisture

	Original Moisture % 17 to 20	
Drying Temperature °F.	150	200
Starch Yield % d.b.	84.6	85.0
Protein in Starch % d.b.	0.39	0.40
Protein in Gluten % d.b.	36.6	37.4

Table V gives the composition of the whole and pearled sorghum, along with the fraction removed by pearling which is referred to as bran.

The samples of whole and pearled grain were processed in the laboratory by the wet-milling procedure illustrated in Fig. 2.

The starch recovered from the pearled grain was whiter than starch made from the whole sorghum. However, it is questionable that this procedure is economically feasible since a substantial quantity of starch is lost in pearling.

Considerable work has been done by F. A. Kummerow *et al.* (8) at Kansas State Agricultural College on the recovery and properties of a carnauba-like wax which is contained in the outside layers of grain sorghums. If this wax can be recovered economically from the bran fraction, it may be profitable to pearl the grain before it is wet milled.

TABLE V

CONSTITUENTS OF WHOLE AND PEARLED MARTIN GRAIN SORGHUM

	Whole	Pearled	Bran
Moisture %	10.0	10.7	8.8
Starch % d.b.	68.9	74.3	35.8
Protein % d.b.	15.0	15.3	—
Oil and Wax % d.b.	3.7	2.8	7.0

Only a few exploratory tests were conducted on the recovery of the wax fraction. Hexane extraction of the bran fraction yielded 5 to 10% of its weight as a crude wax of a brownish green color. Hexane and benzene were the most suitable of the various solvents tried for this purpose. Heptane, iso-heptane, and carbon tetrachloride were difficult to recover. Pigments were removed along with the crude wax when ethyl acetate, or ethyl or iso-propyl alcohols were used. The crude wax upon extraction with acetone yielded a light tan, brittle wax with a melting point of 81°C. A soft fraction consisting mainly of higher alcohols was obtained from the solvent. The brittle wax possibly could be used as a substitute for carnauba wax.

Discussion

It was found that grain sorghum could be processed by a procedure similar to that used industrially for the wet milling of corn. Sorghum starch is not affected by concentrations of sulfur dioxide in steepwater as high as 0.25% at temperatures under 120°F. These are practical operating conditions. It has been shown that grain sorghum can be steeped batchwise in 24 hours. This is a laboratory procedure and the result cannot be transferred directly to plant operation. However,

since corn is steeped satisfactorily in the laboratory in 24 hours, it is assumed that the conditions employed in the countercurrent, multiple contact system for steeping corn on a plant scale will be satisfactory for grain sorghum.

No results have been reported on the recovery of germs from grain sorghum since the operation is almost impossible to conduct quantitatively in the laboratory. However, it was observed that germ recovery was easier and more complete when the concentration of sulfur dioxide in the steepwater exceeded 0.20%.

It appears to be more difficult to separate sorghum starch and gluten than the same materials obtained from corn. It was found that the pitch of the starch table was a critical factor in the preparation of sorghum starch of low protein content. Starch produced from artificially dried grain sorghum contained as little as 0.3% (d.b.) of protein. However, in general, protein contents ranged from 0.4 to 0.7% (d.b.).

If carnauba-like wax can be recovered from grain sorghum economically it may be feasible to pearl the grain before it is wet milled. A whiter starch is obtained and the processing is simplified to some extent since most of the fiber is removed during pearling.

Acknowledgment

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EXTRACTION AND ELECTROPHORETIC ANALYSIS OF THE PROTEINS OF CORN [1]

JOSEPH F. FOSTER, JEN TSI YANG, AND N. HENRY YUI [2]

ABSTRACT

Practically quantitative extraction of the proteins of corn can be effected with dilute aqueous solutions of alkyl benzene sodium sulfonate containing 0.2% bisulfite. In the absence of the reducing agent extraction is limited to about 80%. The whole corn protein extracts are reasonably clear, even following removal of the excess detergent by dialysis against water. The extraction with other media is re-examined, it being found that water removes 11% of the protein, 5% sodium chloride solution 22%, pH 10 carbonate buffer 30%, and pH 7.8 phosphate approximately 20%, all at 2°C. Aqueous ethanol (73%) at room temperature removes approximately 40%. In general extraction with the various media appears to be additive although the yield in the sequence water-salt-carbonate-detergent is not quantitative. Phosphate ion appears to have a very significant insolubilizing action on the proteins, alcohol a somewhat less effect.

Electrophoretic analysis of the detergent extract indicates the presence of at least eight components. Three of these are identified with zein, the others being concentrated in the aqueous extracts.

The question of the overall protein composition of corn is not well clarified. In large part this can be attributed to the fact that the major protein constituent, zein, is soluble only in media which render application of the usual analytical procedures, particularly electrophoretic analysis, difficult or impossible. The amount of zein present and its relationship to the other proteins in corn is in doubt. Thus, it is not clear whether there is a sharp line of demarcation between the proteins or whether there exists a spectrum of proteins ranging from the typical prolamine type to the alkali-soluble glutelins.

It has not heretofore been possible to extract all of the protein of corn in a single extractant or even with a combination of several extractants. Perhaps the nearest approach lies in the work of Nagy, Weidlein and Hixon (4). These workers were able to extract as much as 85% to, in some cases, 90% of the protein of corn using a combination of salt solution, alcohol, and alkali. In this study the workers also observed an unusual solubilizing effect on the corn proteins due to the synthetic detergents. However, they were not able to extract over 60

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to 70% of the protein with the detergent solutions under the conditions they used.

It is now known that zein is soluble in dilute aqueous solutions of soaps and synthetic detergents and the physical chemistry of such solutions has recently been studied by one of us (2), it being shown that in such solutions the zein is present as a very stable zein detergent complex. This raises the possibility of extracting all of the corn protein in a single aqueous medium which could be subjected to electrophoretic analysis.

The present paper demonstrates that substantially quantitative extraction can be attained using dilute aqueous detergent solution buffered at pH 10 containing in addition a small amount of reducing agent such as sodium bisulfite. At least eight electrophoretic components are present. Extraction with such media as water, salt solution, phosphate buffer, carbonate buffer and aqueous alcohol has also been re-examined and electrophoretic analyses made.

Materials and Methods

These studies were carried out on a commercial cornmeal ground to approximately 60 mesh. This sample contained 1.66% Nitrogen on a dry basis corresponding to a protein content of 10.4%. In most of the extraction studies the meal was first defatted by stirring with 99% isopropyl alcohol at room or cold room temperature. Usually 20 gm. samples were stirred twice for approximately 24 hours periods with 100 ml. of the alcohol, and separated by centrifugation. Fat, determined by evaporation of the solvent and weighing of the dry residue, amounted to 4.5–4.7% when extracted at cold room temperature (2°C.) and 5.5 to 5.7% at room temperature. Nitrogen was also determined on the alcohol extracts and amounted to 0.8 to 1.1% of the total nitrogen.

The detergent used was the commercial Santomerse No. 3,[3] an alkyl benzene sulfonate. In some experiments this material was purified by precipitation from ethanol, but with no improvement in extraction.

Extraction Procedure. In early studies extraction was carried out simply by slow mechanical stirring in a beaker. In later work, for reasons mentioned below, shaking was substituted for stirring. In this case the extractions were carried out in 250 ml. centrifuge tubes, the volume of extracting fluid being usually 100 ml., and separation made by centrifugation in an International centrifuge at approximately 2,000 r.p.m. Most extractions were carried out in parallel at room temperature (25–30°C.) and in the cold room (2°C.).

Nitrogen Analysis. The amount of protein extracted was deter-

[3] Supplied by the Monsanto Chemical Co.

mined by nitrogen analysis on the extracts using the Pregl micro technique, with selenium oxychloride as catalyst. Analysis of the corn and of the residue following extraction was by the conventional macro procedure using cupric selenite as a catalyst.

Dialysis. Dialyses were carried out in cellophane tubing[4] using continuous mechanical agitation. For electrophoresis the dialysis time was about 24–48 hours against the appropriate buffer. For removal of excess detergent, dialysis was against five or six changes of water, 12 to 24 hours each. Following dialysis the extracts were concentrated by lyophilization, where necessary, to a protein concentration of at least 0.6% for electrophoretic analysis. In case of the non-detergent extracts solutions were usually somewhat turbid following concentration and centrifugation was frequently necessary to clarify them sufficiently for electrophoretic analysis.

Electrophoretic Analysis. The electrophoretic studies were carried out in a standard double-length cell of the Tiselius type. The apparatus used and method of operation were the same as previously described by one of us (1).

Results and Discussion

Extraction with Aqueous Detergent. General Considerations. The first extractions carried out were with unbuffered detergent solution. In such cases the pH of the detergent, about 8.3, dropped to about 7.0 upon contact with the corn. Extraction yields under such conditions were very poor. Furthermore, it had been found in the case of corn gluten that maintainance of the pH above 9.0 greatly facilitated extraction (7). Therefore, the detergent solutions were buffered with sodium carbonate-bicarbonate at a pH of 10 to 11 in all detergent extractions reported in this paper. The actual pH following extraction ranged from about 9.0 to 10.0.

The first phase of the investigation was concerned with the optimal mechanical conditions for rapid extraction. In runs Nos. 6 and 7 (Fig. 1), 38.0 gm. samples of corn were extracted with a single batch of 200 ml. of 1% detergent solution using vigorous mechanical stirring. Aliquots were removed each day, centrifuged, and the supernates analyzed for nitrogen. Extraction is evidently very slow after the first day. In runs Nos. 8 and 9 (Fig. 1), 18 gm. samples were extracted with 100 ml. portions of 1% detergent for 24 hours, with removal of extract and washing of the residual corn with carbonate buffer (pH 10.3) following each such period. This procedure is seen to be somewhat more efficient.

In runs Nos. 13 and 14 (Fig. 1), extraction was first carried out with carbonate buffer (pH 10.3), 38.0 gm. samples being twice extracted

[4] Supplied by The Visking Corp., Chicago.

with 200 ml. of buffer each time. Each extraction was followed for two to three days. Following this, the residue was extracted twice with 200 ml. portions of detergent (1%), again for two to three days. In all cases extraction can be considered to be substantially complete after 24 hours. Furthermore, the second portion of a given extractant accomplishes relatively little, except in the case of the second extraction with alkaline buffer at room temperature.

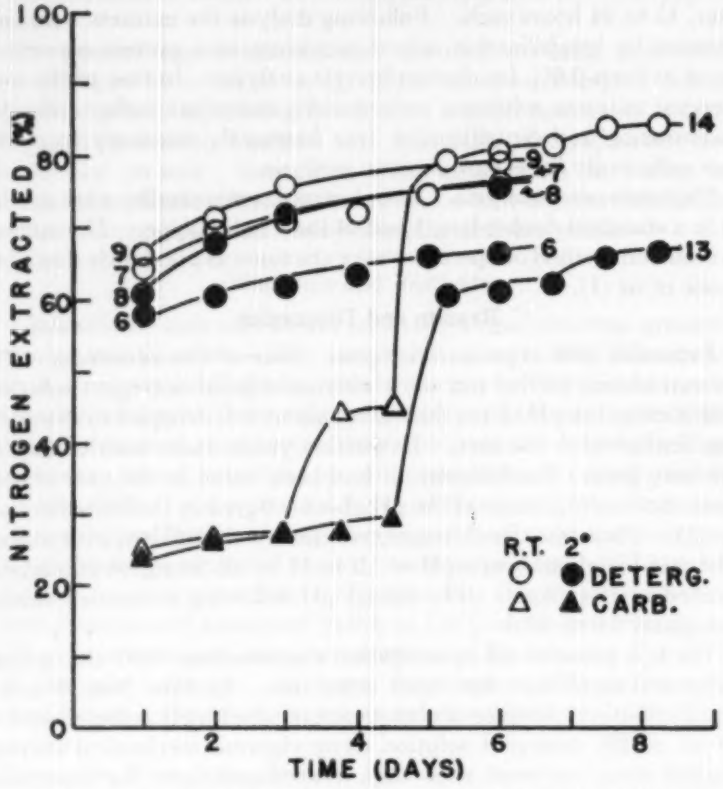

FIG. 1. Extractions with detergent and with carbonate buffer without bisulfite.

This anomalous result with carbonate was observed repeatedly. It was concluded that the effect must be due to microbiological action since it occurred only at room temperature and did not occur in the detergent extractions. In a few extreme cases obvious evidences of deterioration, such as the odor of hydrogen sulfide, were detected. Addition of an effective antiseptic minimized this effect although there were always some doubts as to the validity of results carried out in

alkaline, detergent-free media at room temperature over a period of several days. The antiseptic used in all room temperature work reported below, with the exception of detergent extractions where no evidences of decomposition were ever observed, was *p*-chlorothymol (usually in the amount of 5 ml. of a saturated aqueous solution per 100 ml. of extracting solution).

In the early extractions the extracts were frequently very cloudy following centrifugation. It was concluded that this was in part due to fat, in part due to suspended starch particles. Substitution of either an end to end agitation of the tubes in a horizontal position or end over end rotation yielded equally rapid extraction and much clearer extracts. Use of defatted corn further improved the situation. All subsequent results were obtained with these modifications.

Extraction with Aqueous Detergent Solution. It is apparent from Fig. 1 that extraction with buffered detergent solution or even with alkaline buffer followed by detergent, does not effect quantitative removal of the proteins. The curves appear to level off below 80% extraction and attainment of even this yield is a slow, tedious process. Furthermore, the extracts in these studies were very cloudy, as previously mentioned, and clarification resulted in a loss of as much as 20% of the extracted nitrogen so that even the 70 or 80% figure is high.

Attempts were made to extract the residual nitrogen using aqueous alcohol with and without added sodium acetate at room temperature and at elevated temperature. At room temperature only some 2.0% additional nitrogen could be extracted, at 60° somewhat more; however, the total yields were still not over 80%.

Detergent Plus Reducing Agent. The low yields of protein obtained with the alkaline detergent solutions were distinctly disappointing, particularly in view of the fact that prior to this it had been found possible to extract the protein of commercial corn gluten quantitatively with such a medium (6). It seemed possible that this difference in extractability might be due to the reducing action of the bisulfite steep used in the commercial wet-milling process. Also it is well-known that reducing media facilitate the solubilization of other tissue proteins, notably keratins (3). Therefore, experiments were conducted in which sodium bisulfite in the amount of 0.2% was added to the detergent extractant solutions.

Some results of direct detergent extraction in the presence of bisulfite are shown in Fig. 2. In runs Nos. 29 and 30, 20 gm. samples of defatted corn were extracted repeatedly for 2 day periods with 100 ml. portions of 2.0% detergent containing also 0.2% bisulfite. In runs Nos. 33 and 34, conditions were the same except that the detergent solution used was only about 0.4% (saturated at the cold room tempera-

ture). Runs Nos. 29 and 33 were in the cold room, Nos. 30 and 34 at room temperature. Extraction is obviously much improved and seems to approach 100%.

In Fig. 3 are shown results of experiments in which detergent extraction followed either water or alkaline buffer extraction. In all of these runs conditions were the same during the detergent extractions, i.e., 20 gm. corn extracted repeatedly for 48 hour periods with 100 ml.

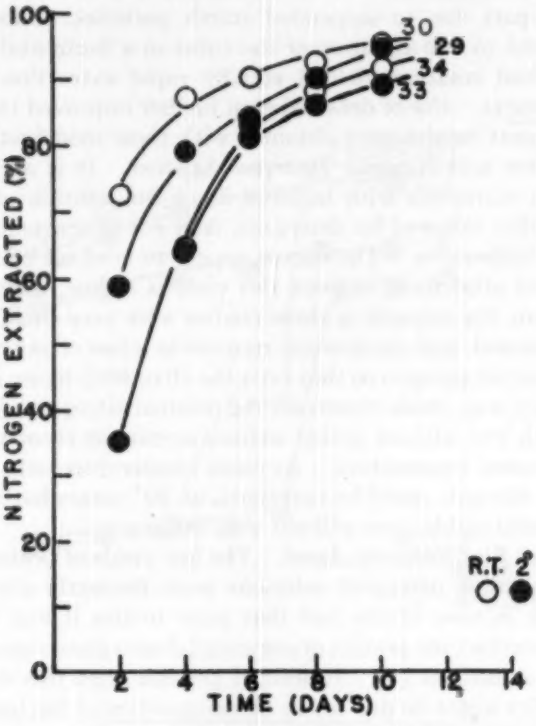

FIG. 2. Extractions with detergent plus bisulfite.

portions of 2% detergent plus 0.2% bisulfite. Removal of the water or alkali soluble protein appears to have no marked influence on the course of the detergent extraction.

The fact that much less nitrogen is extracted in the early stages of run No. 33 as compared to No. 29 (Fig. 2) shows that the excess undissolved detergent in the cold room extractions is effective. This might be expected since presumably the excess dissolves to maintain a saturated system as detergent is combined by protein. At the end of five extractions (10 days) the amount of nitrogen extracted in runs Nos. 33

and 34 is comparable to that in Nos. 29 and 30 in spite of the fact that only 20% as much detergent is used in Nos. 33 and 34. This is of considerable interest and indicates that the ratio of detergent to protein is the limiting factor. Thus the amount extracted in extractions Nos. 1 and 2 is limited by the low detergent concentration in runs Nos. 33 and 34 but is made up in later extractions.

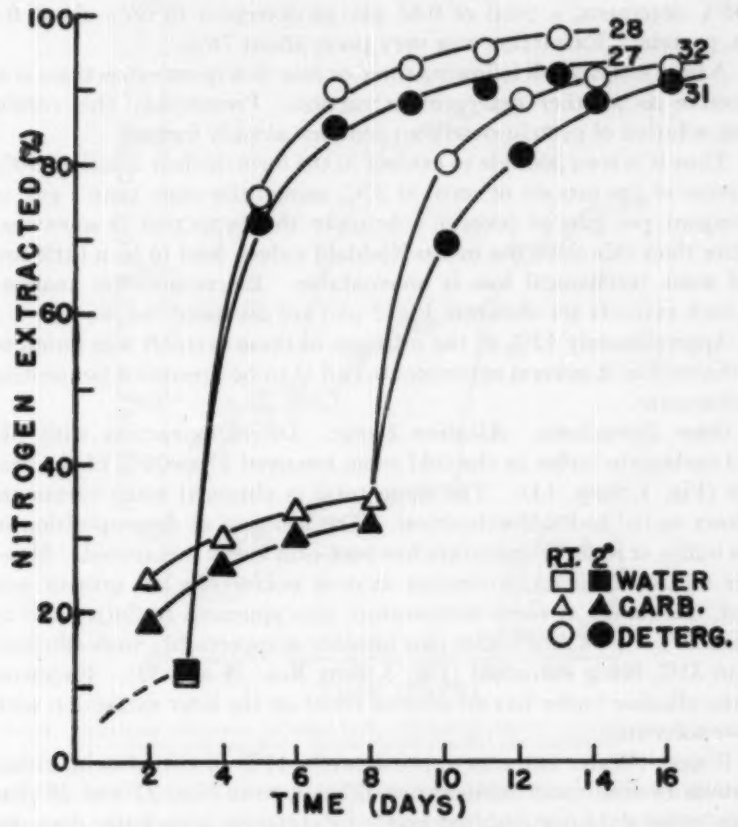

FIG. 3. Extractions with water or carbonate buffer followed by detergent. Bisulfite present in detergent and carbonate extractions.

This result suggested the possibility of reducing the detergent concentration in successive extractions as a means of economizing on detergent without sacrificing on yield or extraction rate. This proved perfectly feasible. In one such experiment a 20 gm. sample of corn was extracted five times as in run No. 29 except that the extracting medium consisted of 1.0, 0.5, 0.3, 0.2, and 0.1% detergent. The

protein extracted was actually slightly higher than in run No. 29. Extraction was 90.0% complete after four extractions. In other words 1.8 gm. of protein was extracted with only 2.0 gm. of detergent. In this experiment the bisulfite concentration was also reduced in successive extractions, from 0.10% down to 0.01%. The ratio of detergent to protein is not the sole factor, however. In a parallel experiment to this a sample of only 5 gm. was extracted with 0.3, 0.2, 0.1, and 0.05% detergent, a total of 0.65 gm. of detergent to only about 0.5 gm. protein. Extraction was very poor, about 76%.

A final water wash following three or four detergent extractions is as effective as another detergent extraction. Presumably this results from solution of protein-detergent complex already formed.

Thus it is seen possible to extract in the form of clear solutions 90% or more of the protein of corn, at 2°C. using little more than 1 gm. of detergent per gm. of protein. Actually the extraction is somewhat better than this since the micro-Kjeldahl values tend to be a little low and some mechanical loss is unavoidable. Electrophoretic analyses on such extracts are shown in Fig. 5 and are discussed below.

Approximately 12% of the nitrogen in these extracts was found to be dialyzable in several experiments and is to be presumed nonprotein in character.

Other Extractants. Alkaline Buffer. Direct extraction with pH 10.3 carbonate buffer in the cold room removed 27 to 29% of the protein (Fig. 1, Exp. 13). The same total is obtained when carbonate follows water and salt extraction. The problem of decomposition in this buffer at room temperature has been previously mentioned. However where adequate protection against microbiological growth was used, the curves at room temperature also appeared to flatten out at about 30%. Alkaline buffer plus bisulfite is appreciably more efficient, 30 to 35% being extracted (Fig. 3, runs Nos. 31 and 32). Exposure to the alkaline buffer has no adverse effect on the later extraction with other solvents.

Water. Water extracts approximately 11% of the protein, either at room or cold room temperature (Fig. 3, runs Nos. 27 and 28 plus many other data not reported here). Extraction with water does not have any adverse effect upon later extractions with other media.

Salt Solution. Extraction with 5% aqueous sodium chloride yields 22% of the protein, following water extraction approximately 11% (a total of 22% with the two extractants). Carbonate buffer following water and salt extraction removes an additional 4.9%, the total agreeing with that obtained by direct alkaline buffer extraction. As seen in Fig. 4, run No. 44, detergent extraction following water, salt, and carbonate is not very effective. As previously pointed out neither water nor carbonate alone interfere with subsequent detergent extraction and

it has also been found that salt followed by detergent gives substantially quantitative extraction.

Phosphate Buffer. Direct extraction with phosphate buffer removes only about 20% of the protein (Fig. 4, runs Nos. 21 and 22). Furthermore the residual protein is rendered much less soluble as indicated by the fact that alkaline buffer following phosphate removes only about 3

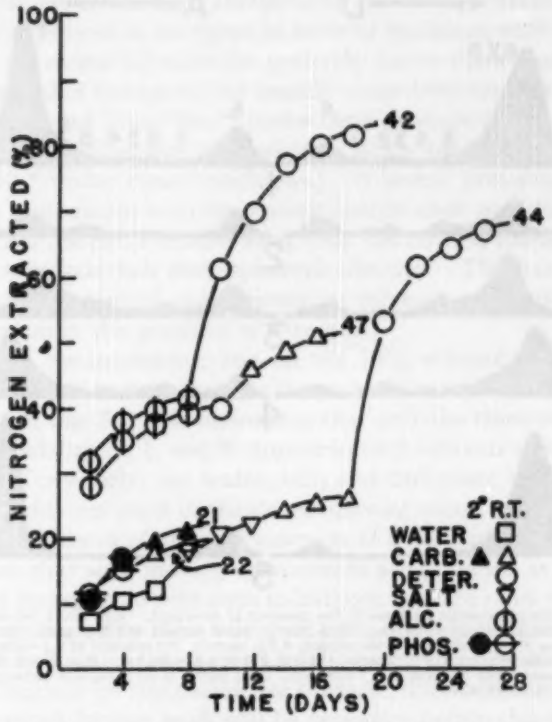

FIG. 4. Extractions with water, carbonate buffer, 5% sodium chloride, 73% ethanol, phosphate buffer, and detergent. Bisulfite present in detergent extraction.

to 5.0% additional protein and extraction with other media is greatly reduced. Detergent without reducing agent following phosphate plus alkaline buffer yielded only 28%. Even with reducing agent extraction is far from complete once the corn has been exposed to phosphate. Exhaustive washing of the residue with water to remove phosphate ions improved this situation only slightly. It appears that phosphate may have an insolubilizing action on the corn proteins.

Aqueous Alcohol. Direct extraction with aqueous alcohol yields about 40% of the protein at room temperature in the presence of sodium acetate. This is in agreement with the findings of Hixon *et al.* (4). More can be extracted at elevated temperature. Extraction with

alcohol somewhat inhibits subsequent extraction with detergent (Fig. 4, run No. 42). Also extraction with alkaline buffer following alcohol appears to be inhibited (Fig. 4, run No. 47). This effect is probably merely one of rate since these curves have not leveled off. In at least one experiment it was found possible to extract a total of over 90%

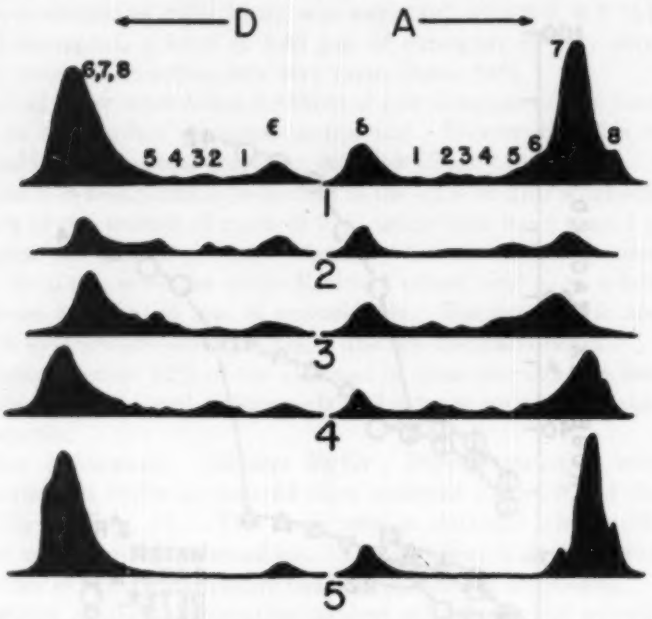

FIG. 5. Electrophoretic analyses in the presence of detergent. Run No. 1, whole-protein, 1.0% protein, 135 minutes at 6.3 volts/cm. Run No. 2, water extract, 0.4% protein, 106 minutes at 6.0 volts/cm. Run No. 3, sodium chloride extract, 0.5% protein, 102 minutes at 6.1 volts/cm. Run No. 4, carbonate buffer extract, 0.6% protein, 135 minutes at 6.4 volts/cm. Run No. 5, 73% ethanol extract, 0.8% protein, 143 minutes at 6.1 volts/cm. The buffer in all cases was carbonate-bicarbonate, pH 10.3, ionic strength 0.26.

of the protein with alcohol followed by detergent. However, it was also noted that such extracts were turbid whereas the direct detergent extracts were quite clear. Thus it seems certain that the alcohol has an insolubilizing action on some of the proteins present in corn.

Electrophoretic Analysis of Extracts. Electrophoretic analyses of water, salt and carbonate buffer extracts have been carried out. The extracts are always very inhomogeneous, containing components ranging in mobility from about 2–3 to 10×10^{-5} cm.^{2} Volt^{-1} Sec.$^{-1}$. It is concluded that such extractions are not particularly selective. Furthermore the analyses are complicated in that the extracts, being very dilute, must be concentrated prior to electrophoresis and there is invariably loss of some protein during this step.

The detergent extracts remain clear following concentration and can be subjected to electrophoresis conveniently. However, in view of the known tendency of proteins to form complexes with anionic detergents the results can be interpreted only with extreme caution (2,5). Results of a typical run on the whole-protein extract from which excess detergent has been removed by prolonged dialysis are shown in Fig. 5. Usually about eight components are distinguishable and these have been numbered in the figure in order of increasing mobility. The mobilities are somewhat variable, probably due to variations in the extent of removal of detergent, but usually range from about 3.0 to about $11–12 \times 10^{-5}$ cm.^{2} Volt^{-1} Sec.$^{-1}$ under conditions similar to those used in Fig. 5. (The detergent itself yields a single peak with mobility of $16–18 \times 10^{-5}$ under these conditions.) It seems probable that this represents a minimum number of components since combination with detergent has the effect of increasing total net charge, thereby causing the proteins to lose their electrophoretic identity. This has been verified by suitable control experiments on mixtures such as zein plus ovalbumin run in the presence of detergent.

A typical electrophoretic run on the 73% ethanol extract under conditions similar to those used in the case of the whole protein is also reproduced in Fig. 5. It is interesting that only the three components of highest mobility (6, 7, and 8) appear in such extracts in appreciable quantity. Conversely, the water, salt, and carbonate buffer extracts are considerably enriched in the slower moving components when analyzed in the presence of minimal amounts of detergent (Fig. 5).

The fact that slow moving components are obtained at all, in the presence of detergent, would seem to indicate that the more water soluble components give up their detergent upon dialysis more readily than zein. If this is true, the method might have value as an empirical analytical method for comparing, for example, different strains of corn. Obviously much further work will be necessary before this is possible. In particular precision methods for determining the residual bound detergent are needed. Further work along these lines is in progress.

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IMPROVED TECHNIQUES FOR STUDY OF THE HYDROLYSIS OF AMYLOSE BY MALT ALPHA-AMYLASE [1,2]

R. J. DIMLER, R. C. BACHMANN AND H. A. DAVIS

ABSTRACT

Amylose solutions of 3 to 4% concentration can be hydrolyzed with malt alpha-amylase without retrogradation if a temperature of 70°C. is used. This high temperature, together with the presence of 0.1% calcium chloride in the amylose solution, minimized possible action of traces of beta-amylase which might have been in the alpha-amylase preparation. The iodine color of the hydrolysis solution reached the dextrin end-point at about 30% conversion and the achromic point at about 45% conversion to apparent maltose. After the hydrolysis was stopped by addition of acetic acid and the solution deionized, the products could be partially fractionated by alcohol precipitation. Paper chromatography showed that the fractions were still very heterogeneous. At the achromic point, the hydrolysis products were found to consist of at least 10 reducing compounds, including glucose and maltose and, presumably, the homologous series of oligosaccharides up to a degree of polymerization of 10 glucose units.

The first study of the hydrolysis of a relatively pure amylose by malt alpha-amylase was described by Meyer and Bernfeld (7). Subsequent work by Myrbäck and coworkers (9, 13) included fractionation by alcohol precipitation of the hydrolysis products at different stages of hydrolysis of the amylose. Data on rates of hydrolysis and on distribution of molecular sizes were used as a basis for theories on the mechanism of alpha-amylase action. Bernfeld and Studer-Pécha (4) have described experiments showing the effect of retrogradation of amylose on the rate and extent of hydrolysis by alpha-amylase. These authors considered the conclusions of Myrbäck unreliable because they were based on experiments in which retrogradation must have occurred (see also Meyer, (8)). The possibility that the fractionation methods used by Myrbäck and coworkers give fractions which are still quite heterogeneous is indicated by the carbon-column adsorption studies of Tiselius and coworkers (16, 17) on fractions furnished by Myrbäck, apparently mainly from starch hydrolyzates.

Our interest in the mode of action of malt alpha-amylase on amylose has led to the development of the methods of operation described

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[2] Contribution from the Northern Regional Research Laboratory, Peoria, Illinois. One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

in this paper. Particular attention was given in this work to methods of minimizing the danger of retrogradation, increasing the usable amylose concentration in order to facilitate the preparation of larger quantities of materials, and evaluating the effectiveness of the fractionation methods.

Materials and Methods

Malt Alpha-Amylase. The preparation of the malt alpha-amylase was based on the work of Kneen, Sandstedt and Hollenbeck (6) on the stability and precipitability of the enzyme. Ground barley malt was extracted with five parts of 0.2% calcium chloride solution at pH 6.0, the filtered extract heated at 70°C. to inactivate beta-amylase, and the alpha-amylase precipitated from the cooled solution by half-saturation with ammonium sulfate. This precipitate was converted to a stable, easily soluble form by dissolving it in 0.2% calcium chloride solution and drying it cryoscopically. The resulting product, representing about 60% of the alpha-amylase originally extracted from the malt, usually had a potency of about 3,000 units per gram by the Sandstedt, Kneen and Blish (11) method.

Amylose. The amylose was prepared from potato starch by the butanol fractionation method of Schoch (12). The recrystallized amylose had an iodine sorption of 201 mg. of iodine per gram by the potentiometric titration method of Bates, French and Rundle (2) as modified by Wilson, Schoch and Hudson (18). In comparison, the maximum iodine affinity of repeatedly recrystallized potato amylose was about 210 mg. of iodine per gram. The amylose either was used as the wet amylose-butanol complex or was dried in such a way as to avoid retrograded or horny products.

Analytical Methods. Reducing power was determined by the method of Somogyi (14). The results were calculated as maltose hydrate.

The qualitative test for butanol in aqueous solution depended upon formation of butyl acetate which was detected by its odor. To a mixture of 2 ml. of the aqueous solution and one drop of glacial acetic acid was added 1 ml. of concentrated sulfuric acid. The heat of mixing aids in the esterification of the butanol and in the volatilization of the butyl acetate. As little as about 0.25% butanol could be detected in this way.

The color of the hydrolyzate with iodine was determined by using the iodine solution and the proportions prescribed for the Sandstedt, Kneen and Blish alpha-amylase determination (11). Measurements were made with a Coleman spectrophotometer, the comparison being either with the iodine blank at 500 mμ or with the inorganic standard of Olson, Evans and Dickson (10) at 635 mμ.

Qualitative analysis of fractions by paper chromatography was done by the multiple development method of Jeanes, Wise and Dimler (5), using a 1:1:1 mixture of fusel oil, pyridine, and water and four ascending movements of the solvent. The position of the sugars was determined by spraying the dried paper with an alkaline solution of 3,5-dinitrosalicylic acid. The initial spot contained about 0.4 mg. of carbohydrate, placed on the paper by application of the necessary number of 1-λ drops to the same spot with drying between each application.

Preparation of Amylose Solution. A 3% solution of potato amylose was prepared by adding a slurry of 60 gm. amylose (dry basis) in 400 ml. *n*-butanol with stirring to 1.6 l. of 0.1% calcium chloride solution at 90°C. To eliminate the possibility of "skin formation" during removal of the butanol it is advantageous to have the solution in a 3-necked, 3-l. round-bottom flask fitted with a distilling head, a stirrer, and a graduated dropping funnel. When solution was complete the butanol was removed by distillation until a negative test for butanol was obtained in the distillate. This usually required distillation of about 1 l. of the mixture. Nearly complete removal of butanol is essential to avoid inactivation of the alpha-amylase. The volume of solution in the flask was kept constant by the continuous addition of water during the distillation.

Hydrolysis of Amylose. The flask containing the aqueous solution of amylose (pH about 6.0) was transferred to a 70°C. constant temperature bath. When the temperature of the stirred solution had dropped to between 70° and 75° the alpha-amylase, dissolved in 0.2% calcium chloride, adjusted to pH 6.3 with dilute ammonium hydroxide and preheated 3 minutes in the 70°C. bath, was added. As an example, a total of 10 units (Sandstedt, Kneen and Blish) of amylase per gram of amylose was used, about half of the enzyme being added initially, one-third after 15 minutes, and the remainder in two equal portions at 15-minute intervals. In this case the achromic point was reached in about 70 minutes. Hydrolysis was stopped by adding 100 ml. of 0.1 *N* acetic acid and holding the acidified (pH 4) solution at 70°C. for 20 minutes before cooling it in an ice bath. The incremental addition of enzyme was used in most cases to more or less compensate for some inactivation of the alpha-amylase at 70°C. Addition of all the enzyme at the beginning of the hydrolysis gave similar results except for requiring about 135 minutes to reach the achromic point.

Preliminary Alcohol Fractionation of Products. The cold hydrolyzate, after being supercentrifuged to remove a slight precipitate, was deionized by passage through columns of cation and anion exchange resins. The solution was concentrated to 180 ml. and added very

rapidly to 3.4 l. of absolute ethanol with stirring to precipitate a fraction insoluble in 95% (v/v) ethanol. After the mixture had been stirred for 18 hours to insure equilibrium, the precipitate was separated by decantation and centrifugation. This fraction was dissolved in 180 ml. of water and reprecipitated from 95% ethanol. It was then fractionated in 85% (v/v) ethanol by dissolving the centrifuged or filtered precipitate in 180 ml. of water and adding it to 1 l. of absolute ethanol. After being stirred overnight the slightly cloudy supernatant was decanted from the sirup which had separated. The fraction insoluble in 85% ethanol was obtained as a dry powder by dissolving the sirup in 90 ml. of water, adding the solution to 3 l. of absolute ethanol with stirring, separating the precipitate by filtration, washing it twice by resuspension in absolute ethanol, and drying it over calcium chloride in a vacuum desiccator. The fraction soluble in 85% and insoluble in 95% ethanol was isolated by concentrating the solution in 85% ethanol to dryness and converting the residue to a dry powder in the same manner used for the fraction insoluble in 85% ethanol. The fraction soluble in 95% ethanol was obtained as a sirup by concentrating the combined 95% ethanol solutions.

Results and Discussion

A difficulty often encountered in working with solutions of amylose is the ease with which retrogradation occurs. In the present studies the relatively good stability of alpha-amylase at high temperatures permitted taking advantage of the fact that the rate at which amylose retrogrades decreases with increase in temperature. In earlier hydrolyses at 30° to 40°C. even 1% solutions frequently retrograded. At 70°C. the use of 3% solutions of amylose proved quite practicable, while some trials were successful with 4%, and slightly higher, concentrations. No cloudiness attributable to retrogradation was observed, although the possibility of formation of invisible submicrons (4, 8) was not excluded.

Retrogradation presumably could be minimized also by use of the procedure described by Bernfeld and Gürtler (3) for hydrolysis with beta-amylase. They added an alkaline solution of amylose to a buffered solution of an excess of enzyme. Such a procedure would not be suitable for the study of intermediate stages of hydrolysis, however, because of the differences in the period of hydrolysis of the various portions of the amylose solution.

With the use of 70°C. for the hydrolysis, the inclusion of calcium chloride for stabilization of the alpha-amylase (6) became desirable. This combination of high temperature and presence of calcium chloride offers the further advantage of providing unfavorable conditions for

beta-amylase (6), thereby minimizing the possibilities of hydrolytic action by traces of beta-amylase which might have remained in the alpha-amylase preparations.

The hydrolysis at 70°C. followed essentially the same course of increase in reducing power as reported by Myrbäck for malt alpha-amylase and by Alfin and Caldwell (1) and by Swanson (15) for pancreatic and salivary alpha-amylase, respectively. The initial rapid increase in reducing power was followed, after about 40–45% conversion to apparent maltose, by a much slower hydrolysis.

The progress of the hydrolysis during the last part of the rapid phase was conveniently followed by determining the color given by the addition of samples of the hydrolyzate to dilute iodine solution. The achromic point occurred regularly at about 45% conversion to apparent maltose. The spectrophotometrically measured transmission of the mixture at 500 mμ became constant at about 95% of the transmission of the iodine blank, apparently as a result of interaction between the iodine and substances in the enzyme preparation.

Another familiar point in the series of iodine colors is the end-point of the alpha-amylase determination (11) at which a red coloration is obtained. The extent of hydrolysis was approximately 30% when the transmission of the iodine-hydrolyzate mixture was the same as that of the inorganic standard (10) at 635 mμ. The test with iodine, therefore, provides a very useful guide for determining when to stop the reaction for these two approximate extents of hydrolysis in the phase of rapid hydrolysis.

Acidification of the hydrolysis mixture was adopted as a more instantaneous method of stopping the reaction than heating the mixture to boiling. This is particularly true when several liters of solution are involved and when the hydrolysis is to be halted at intermediate points in the rapid phase.

The acetic acid was removed along with the calcium chloride by the ion exchange resins. The removal of calcium chloride before alcohol precipitation of the products was found to be essential. Notwithstanding the solubility of calcium chloride in alcohol, the precipitated carbohydrates contained considerable ash if the solution had not been deionized.

TABLE I

Fraction	*Yield* %	*Average D. P.*[1] *glucose units*
Sol. 95% EtOH	40	2.8
Insol. 95%, Sol. 85% EtOH	16	6.3
Insol. both 95% and 85% EtOH	44	7.0

[1] D. P. indicates Degree of Polymerization or number of glucose units per molecule.

Fractionation of the hydrolyzate by precipitation with 95% and 85% (v/v) ethanol was satisfactory only for a preliminary separation of the products into somewhat less complex mixtures. The results shown in Table I are for such a fractionation of the products from a hydrolysis which had been carried to just past the achromic point and

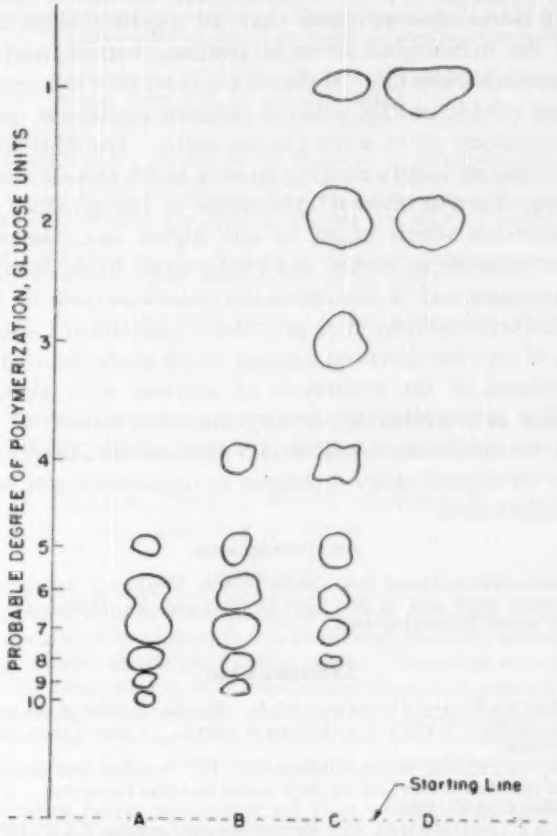

FIG. 1. Paper chromatogram of (A) fraction of amylose hydrolyzate insoluble in both 85% and 95% ethanol, (B) fraction soluble in 85% and insoluble in 95% ethanol, (C) fraction soluble in 95% ethanol, and (D) glucose-maltose mixture. The component sugars are tentatively considered to constitute the homologous series of alpha-1,4-linked glucose polymers as indicated.

showed a reducing power equivalent to 47% conversion to maltose. The yields are only approximate, since they are calculated as percentage of the total weight recovered, which was about 75% of theory. The average degrees of polymerization (D.P.) were calculated from the reducing power, using the reducing power of maltose as a standard. The fraction soluble in 95% ethanol contained, as would be expected,

nearly all of the glucose and maltose formed in the hydrolysis. The fraction insoluble in both 95% and 85% ethanol was essentially free of maltose.

Paper chromatography of these fractions showed clearly that they were all very heterogeneous (Fig. 1). Each contained at least four or five constituents in appreciable amounts, and small quantities of others. It seems most probable that, all together, these compounds constitute the homologous series of glucose, maltose, and alpha-1,4-linked oligosaccharides (5). If that is the case, then it is apparent that the fraction soluble in 95% ethanol contains significant quantities of dextrins containing up to seven glucose units. The least soluble fraction then contains sugars ranging from 4 to 10 glucose units in size. Some chromatograms showed indications of the presence of slower-moving materials which might be still higher saccharides. Studies are now in progress to isolate and characterize more definitely these component sugars and to determine the quantities present.

Paper chromatography thus provides a convenient method for the evaluation of any fractionation method which might be used in studies of the products of the hydrolysis of amylose with alpha-amylase. While similar information apparently can be obtained by the use of interferometric methods in conjunction with carbon column adsorption (16), paper chromatography is simpler in requirements of both equipment and operations.

Acknowledgments

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THE EFFECTS OF CHLORINE DIOXIDE ON THE NUTRITIVE VALUE OF WHEAT GLUTEN [1,2]

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ABSTRACT

The nutritive value of wheat gluten, determined in puppies or in adult dogs, was not altered by treating flour with chlorine dioxide in amounts up to 1.83 gm. chlorine dioxide per hundred weight. There was no evidence of running fits, either clinical, or in electroencephalograms in dogs fed chlorine dioxide-treated wheat gluten. Abnormal electroencephalograms were obtained from dogs fed wheat gluten prepared from flour treated with nitrogen trichloride long before clinical symptoms of abnormalities appeared.

Recent studies have demonstrated that wheat flour treated with nitrogen trichloride, or the gluten therefrom, will produce toxic symptoms when fed to dogs in sufficient quantities. These symptoms are usually described clinically as "running fits" or "canine hysteria" (1–5) and they are associated with an abnormal electroencephalogram (6, 7).

Nitrogen trichloride as a bleaching agent and flour-maturing agent has been superseded by chlorine dioxide. Newell *et al.* (8) have fed dogs, rabbits, rats, and monkeys, flour treated with chlorine dioxide

[1] Manuscript received May 16, 1950.
[2] Contribution from the Bureau of Biological Research, Rutgers University, New Brunswick, N. J.

for periods up to five and one-half months without producing any abnormal symptoms. Humans fed this treated flour for six weeks were also unaffected. Nakamura and Morris (9) have reported that the feeding of chlorine dioxide-treated flour to either growing or mature dogs did not induce canine hysteria.

The following studies were planned to determine whether or not the treatment of wheat gluten with chlorine dioxide would alter the nutritive quality of the protein or produce changes in the electroencephalograms of dogs upon feeding.

Materials and Methods

Six twelve week old littermate puppies were fed the diet in Table I *ad lib* (10). The puppies were paired so that one female and one male were fed the same sample of wheat gluten.

TABLE I

DIET [1]

Wheat Gluten Diet—*ad lib.*			Vitamin Supplements	
Ingredients	Calories	Grams		mg./kg./day
	%	%		
Wheat gluten	21.93	25.0	Thiamine	0.025
Dextrose	31.77	36.2	Riboflavin	.025
Dextrin	16.30	18.6	Nicotinic acid	.250
Lard	30.00	15.2	Calcium pantothenate	.200
Salt Mixture[2]		2.0	Pyridoxine	.015
Agar		3.0	Choline	15.0
			2-methyl-1,4-naptho-quinone	.00001
	100.00	100.0	Calcium tocopherol monosuccinate	0.5
			Fish liver oil	470 Units A 85 Units D
			Liver extract	0.02 USP Units

[1] Mix 1.4 gm. of water with every gram of dog food.
[2] Wesson's Modified Osborne-Mendel Salt Mixture (11).

Nitrogen balance indexes of the gluten were determined on adult dogs according to methods described previously (12). The gluten was water washed from official mill samples of flour containing 65% winter and 35% spring flour. The gluten was prepared in the laboratories of Wallace and Tiernan Company. One sample of flour was untreated, a second was treated with 0.61 gm. of chlorine dioxide per hundred weight, and a third with 1.83 gm. of chlorine dioxide per hundred weight.

Another series of three puppies were fed the wheat gluten from the untreated flour. Electroencephalograms were obtained on these dogs

during a control period and after three and eight weeks feeding of the diet containing this untreated wheat gluten. Another group of three puppies were fed the gluten treated with 1.83 gm. of chlorine dioxide per hundred weight. Electroencephalograms were measured on these as on the control puppies.

Other puppies were placed on a wheat gluten which had been prepared from flour treated with nitrogen trichloride equivalent to 3 gm. per hundred weight. This was done to check the development of hysteria and the effect on the electroencephalogram in dogs.

To determine the encephalograms each dog was anesthetized by intravenous injection of sodium pentothal. While the dogs were asleep electrodes were attached to shaved patches on the right and left frontal areas and the ears, the latter being used as reference points.

After testing the electrode resistance, electroencephalographic records were taken for a period of 20–30 minutes on each dog, at approximately two minute intervals.

Results and Discussion

The caloric intakes are recorded in Fig. 1. These data illustrate the gradual reduction in the number of calories/day/kg. of body weight as the dogs grew in size. The intake was the same for both sexes and was not altered by treatment with chlorine dioxide. The data in Fig. 2 illustrate the growth of the puppies on these caloric intakes. Treat-

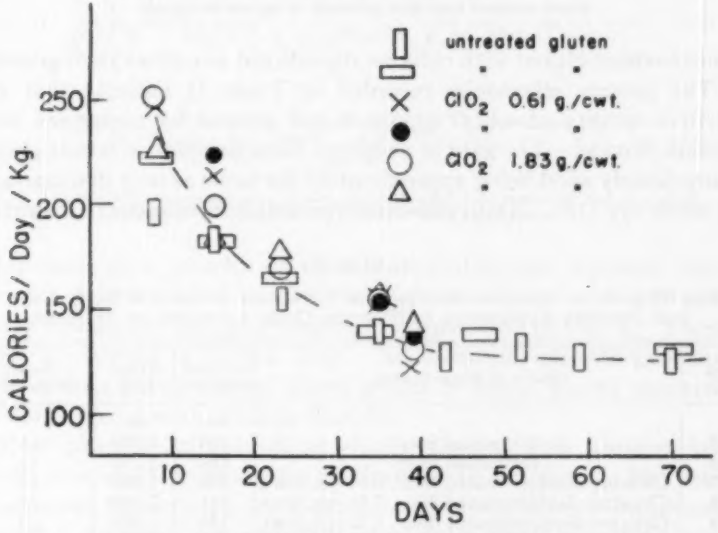

FIG. 1. Decrease in daily calorie intakes of puppies fed diets *ad lib* containing wheat gluten prepared from flour subjected to various treatments.

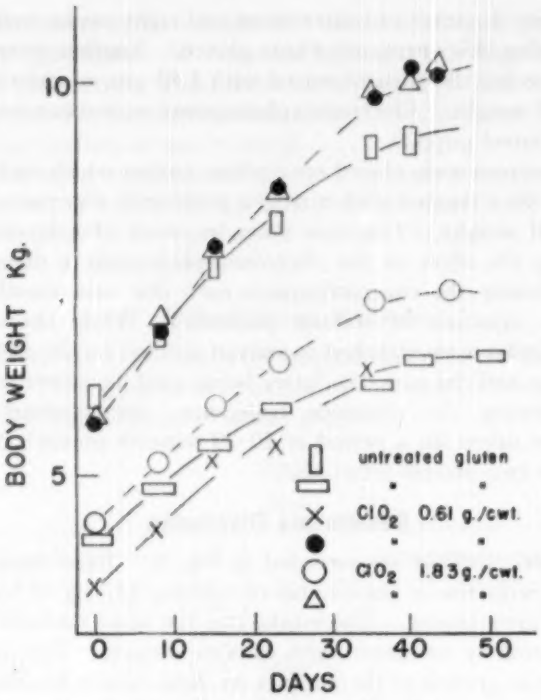

FIG. 2. Growth of puppies fed diet *ad lib* containing wheat gluten prepared from flour subjected to various treatments.

ment of wheat gluten with chlorine dioxide did not effect their growth.

The protein efficiencies recorded in Table II indicate that the nutritive quality of wheat gluten is not altered by treatment with chlorine dioxide. The gain in weight of these puppies on wheat gluten is surprisingly good being approximately the same as on a diet containing whole egg (13). Data presented previously demonstrate, however,

TABLE II

THE EFFECT OF VARIOUS GLUTENS ON NITROGEN INTAKE, WEIGHT GAIN AND PROTEIN EFFICIENCY OF PUPPIES OVER A PERIOD OF 21 DAYS

Dog No.	Source of Wheat Gluten	Nitrogen Intake	Weight Gain	"Protein Efficiency"[1]
		gm.	gm.	
85	Untreated flour	136	1,550	11
86	Untreated flour	180	2,200	12
87	Chlorine dioxide-treated flour, 0.61 gm./cwt.	140	1,800	13
88	Chlorine dioxide-treated flour, 0.61 gm./cwt.	211	2,800	13
89	Chlorine dioxide-treated flour, 1.83 gm./cwt.	156	1,850	12
90	Chlorine dioxide-treated flour, 1.83 gm./cwt.	202	2,700	13

[1] "Protein efficiencies" are grams body weight gain per gram of nitrogen intake during this period.

TABLE III

DIGESTIBILITIES AND NITROGEN BALANCE INDEXES[1] OF WHEAT GLUTEN PREPARED FROM UNTREATED FLOUR AND FROM FLOUR TREATED WITH 1.83 GM. CHLORINE DIOXIDE PER HUNDRED WEIGHT

Source of Wheat Gluten	Digestibility	Absorbed Nitrogen AN	Urinary Nitrogen UN	Protein-free Urinary Nitrogen UN_0	Nitrogen Balance Index K
	%	*gm./day/sq.M.*	*gm./day/sq.M.*	*gm./day/sq.M.*	
Untreated flour	98	4.28	4.03	1.88	0.50±.06[2]
Chlorine dioxide-treated flour, 1.83 gm./cwt.	98	4.24	4.47	2.29	0.49±.05[2]

[1] The values are averages obtained on six adult dogs.
[2] Standard error of mean.

that the growth on whole egg involves a higher protein efficiency, more protein and less fat being laid down with whole egg than with the wheat gluten diets (13).

Digestibilities and nitrogen balance indexes of gluten from untreated and from chlorine dioxide treated flour were determined in adult dogs, the results being recorded in Table III. These data demonstrate further that the digestibility and the retention of nitrogen in the animal is not altered by treating the flour with chlorine dioxide. The indexes of this sample of wheat gluten are slightly higher than the average of 0.44 previously reported for another sample (14).

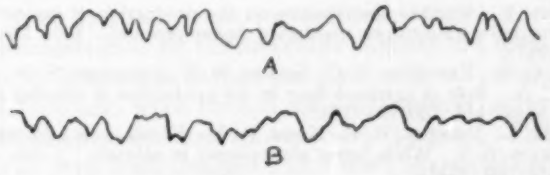

FIG. 3. Typical electroencephalogram in dogs obtained after three weeks (A) and after eight weeks (B) of feeding chlorine dioxide treated wheat gluten.

Representative samples of electroencephalograms obtained after three and eight weeks of feeding wheat gluten treated with 1.83 gm. chlorine dioxide per hundred weight, are illustrated in Fig. 3. These records are typical of normal dogs. No abnormal records were obtained on dogs fed untreated wheat gluten or wheat gluten prepared from flour treated with chlorine dioxide.

Three animals fed the wheat gluten, treated with nitrogen trichloride developed "running fits" within six to ten days. The dogs were removed immediately from the diet after the abnormal symptoms appeared. Recovery was complete when the animals were placed on the control ration. A typical fit pattern from such a dog is illustrated in Fig. 4. The heightened activity illustrated is common with dogs

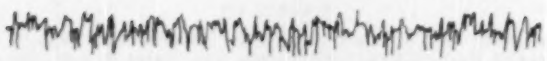

FIG. 4. Typical electroencephalogram in dogs obtained after seven days of feeding wheat gluten treated with nitrogen trichloride.

suffering from running fits and is sometimes recorded in these animals several days before clinical symptoms appear. At no time was this increased activity noted in the electroencephalograms from puppies fed the chlorine dioxide-treated wheat gluten and the clinical symptoms of running fits were entirely absent. It can be concluded, therefore, that dogs fed gluten prepared from flour treated with chlorine dioxide at the level employed in these studies (1.83 gm./cwt.) exhibit no abnormalities in cortical activity.

Acknowledgment

These studies were supported by a grant from the Wallace and Tiernan Company, Inc. Newark, New Jersey.

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INFLUENCE OF BLEACHING ON THE ABSORPTION SPECTRA OF BUTYL ALCOHOL EXTRACTS OF WHEAT FLOUR [1]

MAX MILNER AND W. W. DODGE

ABSTRACT

The spectrophotometric characteristics of water-saturated butyl alcohol extracts of wheat flour treated with various concentrations of chlroine dioxide and nitrogen trichloride, showed that the so-called residual pigment which remains in flour after bleaching with high concentrations of these gases is not a carotenoid. This material, probably lipid in nature, has a primary absorption peak in the ultra-violet region somewhat below 260 mu. In the visible region the effect on this compound of even large dosages of chlorine dioxide, nitrogen trichloride, and benzoyl peroxide was minor. In the ultra-violet spectrum the effect of these agents was marked. Treatment with benzoyl peroxide in amounts used for normal bleaching was most effective in reducing the ultra-violet absorption, whereas very high levels of this oxidizing agent intensified this absorption.

A previous publication (2) dealing with the influence of physical variables on efficiency of flour bleaching with nitrogen trichloride, indicated that massive dosages of this oxidizing gas did not eliminate the light absorption characteristics of water-saturated butyl alcohol extracts of flour samples, when pigment concentration is determined spectrophotometrically by the procedure outlined in Cereal Laboratory Methods (1). Thus, one unbleached flour with a "carotene" concentration of 2.0 p.p.m. when treated with successively larger amounts of this gas showed no color removal beyond 0.65 p.p.m. This color value was retained even with dosages which were approximately 100 times those normally used in commercial bleaching. This heavily overtreated flour had assumed an unnatural pink color, was very rancid, and did not develop a cohesive dough when mixed with water.

The present report deals with a spectrophotometric characterization of the residual pigment and the effect of treatment with nitrogen trichloride, chlorine dioxide, and benzoyl peroxide on the absorption spectrum.

Materials and Methods

Two unbleached flours commercially milled from average mixes of hard red winter wheat were used in this study. Their composition was as follows:

[1] Manuscript received April 21, 1950. Contribution No. 178, Department of Milling Industry, Kansas Agricultural Experiment Station, Manhattan, Kansas. This research was supported by a grant from the Wallace & Tiernan Co., Inc., Newark, New Jersey.

Flour sample	*Protein*[1] %	*Ash*[1] %	*"Carotene"* p.p.m.
A	10.6	0.40	3.3
B	10.4	0.41	2.7

[1] 14% moisture basis.

Bleaching with nitrogen trichloride was carried out by a regular laboratory procedure previously outlined (2) and flour treatment with chlorine dioxide was performed using the acetic anhydride-sodium chlorite method recently described (4). Bleaching with benzoyl peroxide was accomplished by mixing the flour thoroughly with the required amounts of a commercial preparation consisting of 32% benzoyl peroxide in a mixture containing potassium aluminum sulfate and magnesium carbonate as inert diluents.

Extraction of flour pigments with water-saturated N-butyl alcohol was carried out by the procedure outlined in Cereal Laboratory Methods. The absorption spectra of the extracts measured in terms of optical density were determined with the Beckman Photoelectric Quartz Spectrophotometer, over the wave length interval from 260 to

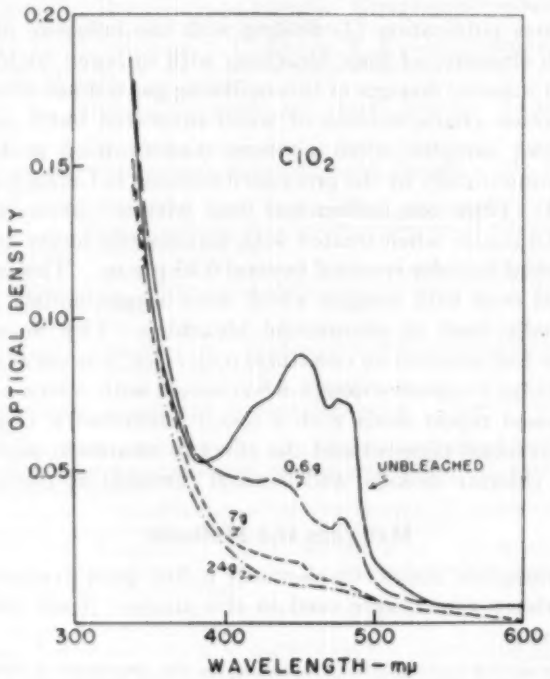

FIG. 1. Influence of various dosages of chlorine dioxide (grams per 100 pounds) on the absorption spectrum of water-saturated butyl alcohol extracts of wheat flour.

600 mu. Readings were taken at intervals not greater than 10 mu throughout the spectral range studied. When operating in the ultra-violet region below 360 mu the mercury vapor radiation source and silica cells were used. For convenience in graphical presentation of the absorption spectra the data obtained in the ultra-violet region below 360 mu are plotted separately from those obtained in the range from 360 to 600 mu.

Results

The absorption spectra of extracts of unbleached flour "A," and of the same flour bleached with various dosages of chlorine dioxide be-

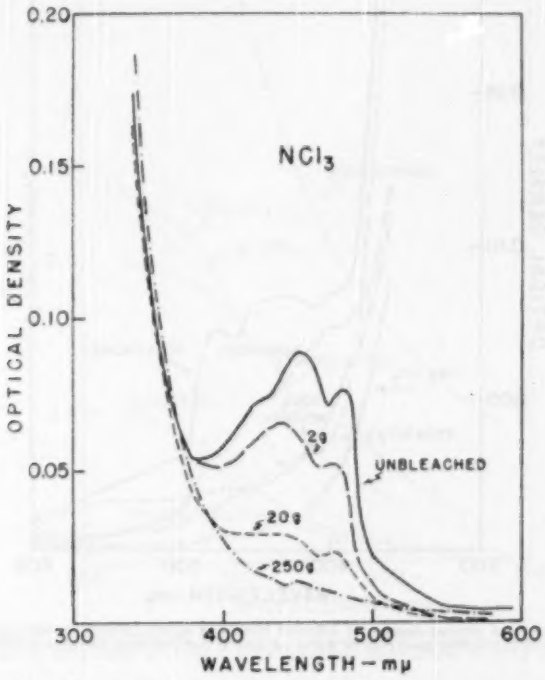

FIG. 2. Influence of various dosages of nitrogen trichloride (grams per 100 pounds) on the absorption spectrum of water-saturated butyl alcohol extracts of wheat flour.

tween 320 and 600 mu, are presented in Fig. 1; similar results obtained with nitrogen trichloride treatment are shown in Fig. 2.

The uppermost curve in both Fig. 1 and Fig. 2 in the range from somewhat below 400 to 600 mu is the typical absorption spectrum of a mixture of xanthophyll pigments, carotene, and other closely related carotenoid materials known to be present in wheat flour. Treatment with increasing concentrations of nitrogen trichloride and chlorine

dioxide, as would be expected, causes a corresponding elimination of this typical spectrum from the flour extracts. It is notable, however, that although the largest dosages of nitrogen trichloride and chlorine dioxide virtually eliminated the spectra due to the carotenoid pigments, the spectrum of another light-absorbing material was revealed, which appeared to be almost unaffected by these oxidizing agents. The absorption intensity of this material increased progressively at wave lengths below 500 mu, and was particularly strong as the ultra-violet

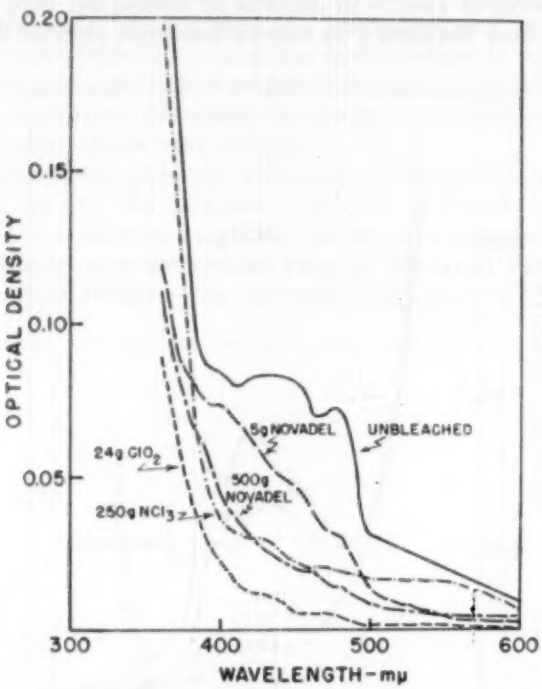

FIG. 3. Influence of various dosages of different bleaching agents (grams per 100 pounds) on the absorption in the visible spectrum of water-saturated butyl alcohol extracts of wheat flour.

region was approached. The spectrum of this compound in the visible range appeared to be unaffected by the very high concentrations of the bleaching gases applied. This absorption was obviously due to the so-called residual pigment in flour which remains after the complete removal of the carotenoids. It exhibited significant light absorption at a wave length of 435.8 mu which is that used in the method for pigments prescribed in Cereal Laboratory Methods (1).

The apparently refractory nature of this residual material in the visible range of the spectrum where the carotenoids absorb most

strongly, suggested that an examination in other regions of the spectrum might disclose alterations due to high treatment rates. For this study the "B" flour was used. This flour was bleached with nitrogen trichloride at the rate of 250 gm./cwt. and with chlorine dioxide at the rate of 24 gm./cwt. These treatments were approximately 100 times the normal commercial levels. In addition, a sample was treated with benzoyl peroxide mixture at the normal rate of 5 gm./cwt. as well as at 500 gm./cwt. The absorption spectra in the visible range between

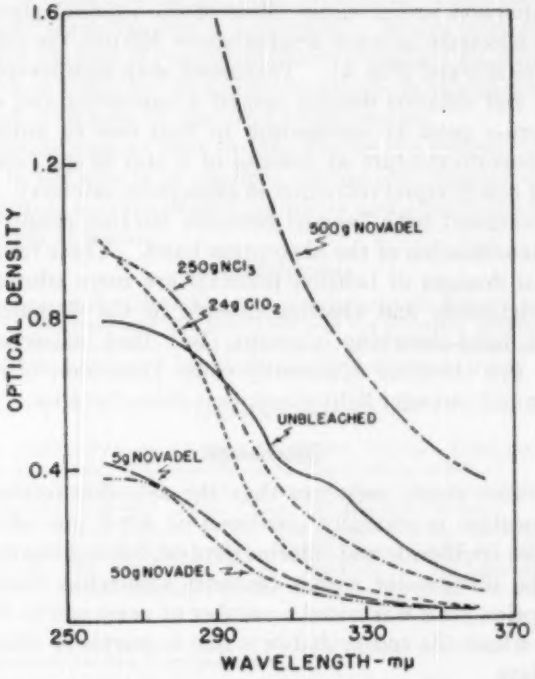

FIG. 4. Influence of various dosages of different bleaching agents (grams per 100 pounds) on the absorption in the ultra-violet spectrum of water-saturated butyl alcohol extracts of wheat flour.

360 and 600 mu of the extracts of the treated and untreated flours prepared several days after treatment are shown in Fig. 3, and the spectra in the ultra-violet region from 250 to 360 mu are shown in Fig. 4. The data in Fig. 4 were obtained by diluting the original extracts in the ratio of 1:4. The absorption characteristics at wave lengths shorter than 260 mu could not be determined due to the strong absorption below this wave length by the solvent itself.

The data of Figs. 3 and 4 indicate that benzoyl peroxide in normal concentrations apparently possesses an efficiency in oxidizing the caro-

tenoid pigments comparable to that of nitrogen trichloride and chlorine dioxide. At the 100-fold normal level of treatment the spectra of the extracts from the benzoyl peroxide and nitrogen trichloride-treated samples are similar as regards shape and intensity when the wave length region characteristic of the carotenoid pigments is considered. However, similar treatment with chlorine dioxide appeared to cause a definite alteration in the absorption band of the residual pigment. In this case the absorption curve was similar in shape but was significantly reduced in intensity.

In comparison to the minor effect of the residual pigment on the absorption spectrum at wave lengths above 360 mu, the changes below 360 mu were marked (Fig. 4). Treatment with high levels of nitrogen trichloride and chlorine dioxide caused a narrowing and elevation of the absorption peak in comparison to that due to untreated flour. Benzoyl peroxide mixture at dosages of 5 and 50 gm./cwt. caused a drastic and nearly equal reduction in absorption intensity, whereas the 500 gm. treatment with benzoyl peroxide mixture resulted in a very marked intensification of the absorption band. These results indicate that normal dosages of benzoyl peroxide are more efficient than are nitrogen trichloride and chlorine dioxide in the destruction of the ultra-violet light-absorbing pigment, but that extremely massive dosages of this chemical apparently cause formation of a derivative which has much stronger light absorption characteristics.

Discussion

The present study indicates that the so-called residual pigment whose absorption is virtually unaltered at 435.8 mu when bleached with nitrogen trichloride and chlorine dioxide, has a primary absorption band in the ultra-violet region centered somewhat below 260 mu. This absorption peak was noted a number of years ago in ethyl alcohol extracts of wheat (3) and probably is due to sterols or other materials related to fats.

It is probable that two effects are responsible for the changes in the absorption spectra observed in the region from 360 to 260 mu. Certain modes of oxidation may produce substances from carotenoid pigments exhibiting low absorption in the region where carotenoid absorption is normally high (440 to 500 mu) and high absorption in the ultra-violet. Similarly, the nature of the oxidant employed may differentially affect the lipid fraction in the two spectral regions. It is probable that nitrogen trichloride and chlorine dioxide which had no detectable influence on the absorption spectrum of the lipid fraction (residual pigment) in the carotenoid absorption range, would also have little effect on this fraction in the ultra-violet region, and that the

changes in spectral characteristics in the latter range are due to carotenoid oxidation products. Similarly benzoyl peroxide in the lower range of concentration (5 gm. and 50 gm.) probably yields carotenoid oxidation products of reduced absorbing power in the ultra-violet region. On the other hand, very high dosage of benzoyl peroxide (500 gm.) probably causes profound oxidative changes in the lipid fraction, yielding the very high absorption noted in this region.[2]

The present studies suggest that the efficiency of benzoyl peroxide as a flour bleaching agent is about the same as that of the gaseous agents, nitrogen trichloride and chlorine dioxide, when the removal of the carotenoid pigments only is considered. It is of interest, nevertheless, that this reagent is considerably more efficient than are the gaseous materials in reducing the radiation-absorbing intensity of materials which absorb primarily in the ultra-violet region.

These results suggest that further studies are needed to clarify the differences in the effects of nitrogen trichloride, chlorine dioxide, and benzoyl peroxide on the isolated pigments and other radiation-absorbing compounds of wheat.

Acknowledgments

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[2] The authors are indebted to G. N. Irvine of the Grain Research Laboratory, Winnipeg, for suggesting the interpretation outlined in this paragraph.

STUDIES ON A RAPID TEST FOR THE VIABILITY OF CORN FOR INDUSTRIAL USE [1,2]

PEGGY D. BAIRD, M. M. MACMASTERS, AND C. E. RIST

ABSTRACT

The 2,3,5-triphenyltetrazolium chloride test for viability is suggested as a means of detecting corn kernels that have been killed by high temperature during drying or by freezing, and for determining the percentage of dead kernels in corn before purchase. The test with the reagent is simple and requires only four hours for completion.

Corn processors have long needed a rapid test to determine, before purchase, the suitability of grain for their use. In the past few years this need has grown as the result of the increasingly prevalent practice of drying corn artificially. Several factors have contributed to make artificial drying a more common practice, chief of which is the present widespread use of mechanical corn pickers. Harvest with a corn picker is completed in about one-third, or less, the length of time formerly required for hand picking. Hence much of the corn has less time to dry in the field.

The number of publications on the structure and use of corn driers is witness to the prevalent need for drying corn before it is stored. No problem would exist if recommendations (3, 4, 10) were heeded, but a tendency to use too high temperatures, both on the farm and at elevators, persists.

Some corn wet millers have been loathe to purchase corn from a county in which a commercial drier was known to be operating because over-heating during drying damages corn for starch production. Processors have stated that dead corn is undesirable for the production of starch. These facts, together with the experience of seed corn producers that over-heating destroys viability, led to the investigation of a test for viability of corn which might serve to indicate the value of the corn for industrial processing. The present data are presented as a progress report.

Materials and Methods

Viability Test. The viability test with 2,3,5-triphenyltetrazolium chloride, which was developed and applied to corn by Lakon (5, 6 ,7), was selected because the reagent gives an easily discerned carmine red

[1] Manuscript received May 19, 1950.
[2] Contribution from the Northern Regional Research Laboratory, Peoria, Illinois. One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

color to living portions of the germ. Lakon and others have described the test as requiring a total of 7 to 18 hours for completion. Our preliminary experiments had shown that the total time required could be reduced to a maximum of about 4 hours. One hundred kernels of the corn to be tested were soaked for from 1 to 2 hours in distilled water. The shorter period was used when soft corn[3] was tested, the longer when naturally or artificially-dried corn was being examined. After soaking, each kernel was bisected longitudinally perpendicular to the broad face. The cut was made with a sharp razor blade while the kernel was held under distilled water. Bisecting under distilled water tended to prevent the formation of a deposit over the cut surface.

One half of each kernel was laid, cut side down, in a petri dish containing sufficient 1% aqueous triphenyltetrazolium chloride solution to cover the kernel pieces. The dish was then placed in a dark cabinet at room temperature for from 1 to 1½ hours. The kernel pieces were then examined to determine viability. The variation between duplicate samples was within the range, 0–5%.

The viable parts are colored carmine red by this procedure. In strongly viable corn, (i.e., kernels which will give vigorous seedlings), the entire germ, composed of the embryonic plant and the scutellum, is colored. There are certain instances, however, when only portions of the germ are colored yet the seed is still viable. Typical staining patterns of kernels bordering between viability and non-viability are shown in Fig. 1. It is these patterns which are most difficult to interpret and which, therefore, require the deepest coloration with the reagent. In such cases, the red color appears (1) in all parts of the scutellum, and in all of the embryonic axis except the radicle (the embryonic root); (2) in only the medial zone of the scutellum, and in all of the embryonic axis (Fig. 1–2); and (3) in only the medial zone of the scutellum, and in all but the radicle of the embryonic axis (Fig. 1–3). If only strongly viable corn is considered to be suitable for use, the period of contact of the kernel pieces with the reagent can be limited to 30 minutes. In this case only those pieces which show a pink color over the entire cut surface of the germ would be counted as viable.

In cases of urgency at least a close estimate of the viability of corn can be made with triphenyltetrazolium chloride in only one hour. The corn is bisected without the preliminary soaking, and the viability is determined after a one-hour staining period. There is some mechanical difficulty in bisecting the hard kernel. The resulting damage to the cells sometimes causes formation of a heavy scum which must be removed before the viability of the kernel is determined.

[3] "The term soft corn can be applied properly to any corn that is soft and watery, in contrast to the relatively hard dry kernels of ears as they are cribbed under ordinary conditions" (9).

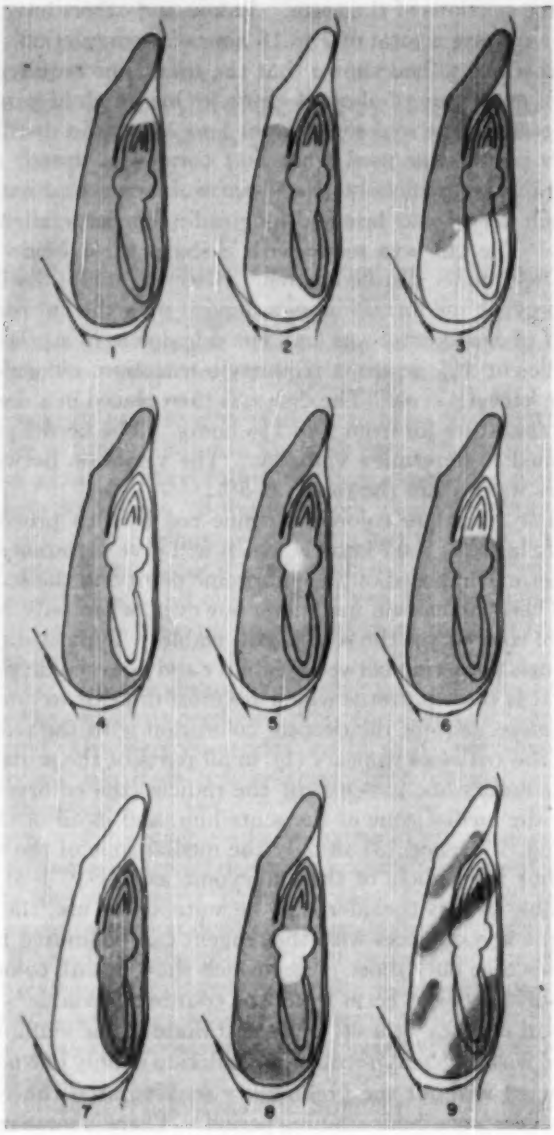

FIG. 1. Typical coloration of corn germ in border-line cases. Shaded portion represents the area colored carmine red by the test.

1. Viable—surface tissues which do not readily take up the reagent exposed by improper bisection.
2. Viable—medial portion of scutellum and embryonic axis colored.
3. Viable—all colored except radicle and lower scutellum.
4. Non-viable—no part of embryonic axis colored.
5. Non-viable—a lateral bud uncolored.
6. Non-viable—plumule uncolored.
7. Non-viable—entire scutellum uncolored.
8. Non-viable—mesocotyl region uncolored.
9. Non-viable—streaked, with no essential part completely colored.

Care must be taken in bisecting the kernels. If the upper portion of the embryonic plant is not cut through, surface tissues may be exposed which do not readily take up the reagent (Fig. 1–1).

In testing corn heavily contaminated with bacteria, a colored deposit may form over the cut surface. This deposit need not interfere with the test; it can be scraped away before the kernel is examined (7).

The reagent solution is sensitive to light, but it can be kept for several months in a dark bottle stored in a refrigerator. It is colorless when prepared; appearance of a dark amber color indicates that it is no longer useful (7).

Corn which has previously been frozen often gives a purplish-red color, rather than the usual carmine red, when treated with the reagent.

Standard germination tests were run on each sample as a standard in judging the accuracy of the triphenyltetrazolium chloride test. One hundred kernels were placed between moist layers of cheesecloth, or similar material, and the precent of kernels germinated was determined at the end of 5 to 7 days. This standard germination test usually agreed with the color test for viability within 10%.

Corn Tested. Four samples of naturally soft, shelled corn, not moldy, containing from 18.6 to 30.3% moisture, were dried immediately upon receipt at several temperatures, at 10° intervals from 110° to 220°F., to approximately 13% moisture content, each in 1 × 5 × 5-inch containers made of quarter-inch wire cloth, which were filled and placed in a forced-draft oven maintained at the required temperature. One sample was also obtained after commercial drying, and one which had been dried artificially on a farm.

Attempts were made to increase the number of samples by tempering naturally-dried corn to a higher moisture content. These were abandoned when it was found that soft corn and tempered corn of the same moisture content behaved dissimilarly as to viability even during short periods of storage.

Processing. The laboratory wet-milling process method used was that described by Cox, MacMasters, and Hilbert (2). In most cases, the steeping solution contained 0.15% sulfur dioxide; more rarely a 0.10% sulfur dioxide solution was used.

Results and Discussion

Under the conditions of drying used, temperatures above approximately 120° to 140°F., depending upon the individual sample, greatly decreased the viability of the corn. Typical data are shown graphically in Fig. 2. A correlation between viability and ease of processing of corn is strongly suggested, since Shedd (10) and Hukill (4) state that corn dried at temperatures above 130°F. is damaged for at least certain types of processing.

It is known, although too often overlooked in drying practice, that the effect of drying temperature upon viability is influenced by the moisture content of the grain (3, 8). This may explain some results which are otherwise anomalous from the standpoint of both viability and ease of processing.

It is impossible to draw final conclusions from the small number of samples which were processed. Certain observations made during the laboratory processing studies suggest possible effects of drying temperature upon the processing behavior of corn. Corn dried at temperatures above approximately 130°F. usually gave harder grits and more

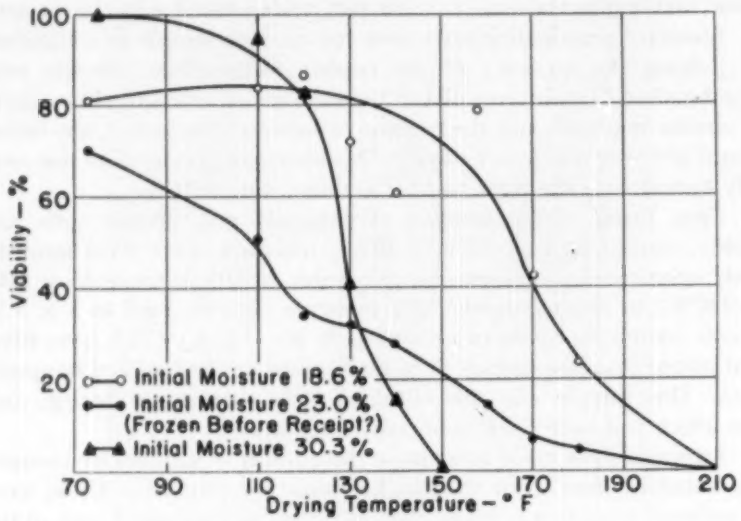

FIG. 2. Effect of drying temperature on viability of corn.

rubbery germ than that dried at lower temperatures. While no germ flotation was attempted, it was noted that the rubbery germs tended to sink when suspended in the ground corn slurry. There was also a marked tendency for oil to be found in starch separated from the over-heated corn. The reported difficulty in the industrial processing of artificially-dried corn may well be attributable, therefore, to the fact that but little attention has been given in the past to control of the temperature when drying corn destined for use other than as seed.

Freezing, as well as over-heating, may decrease the viability of corn. In the present experiments, it was found that corn containing more than about 23% moisture after being frozen, decreased rapidly in viability. At lower moisture contents of the corn, the decrease in viability after

freezing was relatively slow. Development of a purplish-red color during the triphenyltetrazolium chloride test has been observed only in corn which has previously been frozen (cf. 1). Some frozen corn, however, gives a carmine red color with the reagent, hence the test is not specific for the detection of corn damaged by freezing.

There are no figures available on the actual economic disadvantage of processing dead corn. However, industrial experience leads to the definite conclusion that dead corn causes departures from normal milling results. Corn dried at temperatures too high gives poor quality and low yields of starch when wet milled. Here, too, the germ has been killed and the determination of viability may give an indication of milling quality. A means of determining the condition of commercial corn is therefore of direct economic importance.

On the basis of the knowledge at hand, the test for viability with 2,3,5-triphenyltetrazolim chloride solution appears to offer promise for the determination of the industrial suitability of corn as offered for sale. This test requires no complicated equipment, relatively little time, and a minimum of technical knowledge on the part of the operator.

Acknowledgments

The authors gratefully acknowledge the laboratory assistance of Margaret Holzapfel and the help of the Analytical and Physical Chemical Division of this Laboratory in procuring samples and making chemical analyses of the corn.

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COMMUNICATION TO THE EDITOR

Stabilization of Solutions of Wheat Gluten in Dilute Acetic Acid by Brief Heat Treatment

SIR:

Rose and Cook (5) first reported that solutions of wheat gluten in dilute acetic acid are unstable. Evidence for the instability was a drop in viscosity over a period of time. Hydrolytic changes were also measurable (1). Olcott, Sapirstein, and Blish (4) concluded that the changes were catalyzed by a heat-sensitive enzyme, since the solutions were almost completely stabilized by heat treatment (5–10 minutes at 100°). This observation suggested that acetic acid solutions of wheat gluten to be used in research investigations should be heat-treated as soon as possible after their preparation to destroy the enzyme.

A simple set-up for accomplishing this end has been used routinely in this laboratory for the past 8 years (Fig. 1). The rate of flow of the gluten solution is regulated either by means of the clamp or by adjusting the height of the reservoir. In two sets of early experiments it was shown that the gluten solution need be heated for only a very brief period to achieve stabilization. Data are shown in Table I. With the most rapid flow the gluten solution was in the steam chamber only 11 seconds. However, it was not possible, even with the more severe treatments, to stabilize the solutions completely. The cause of the

TABLE I

	Rate of Flow	Time in Steam Chamber	Kinematic Viscosity after Elapsed Time in Days at 25°				Total Decrease
	ml./min.	*min.*	*0*	*1*	*3*	*5*	
Exp. I[1]	Control	—	2.48	1.98	1.66	1.55	0.93
	31	7	2.31	2.29	2.26	2.22	.09
	90	2.3	2.46	2.41	2.35	2.32	.14
	220	1	2.50	2.46	2.41	2.38	.12
Exp. II[2]	Control	—	3.91	3.19	—	2.44	1.47
	17	2.3	3.92	3.72	3.62	3.59	.33
	44	0.91	4.08	3.85	3.71	3.66	.42
	90	0.44	4.18	3.93	3.78	3.74	.44
	210	0.19	4.19	3.91	3.76	3.68	0.51

[1] The original solution contained 4.2% gluten (N × 5.7) in 0.1 *N* acetic acid. After heat treatment, the various samples were diluted with an equal volume of 0.1 *N* acetic acid for the viscosity determinations. Volume of the steam chamber, 210 ml.

[2] Gluten was dissolved in 0.05 *N* acetic acid solution, to make approximately 3.5% solution (pH 4.0). Volume of steam chamber, 40 ml.

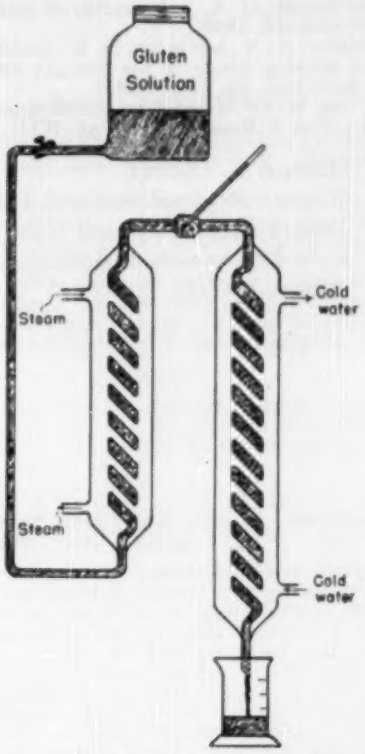

FIG. 1. Apparatus used for continuous rapid heat treatment of gluten solutions.

slow but significant drop in viscosity of heated solutions is not known. Microbiological contamination was suspected but it was found that no organisms could be detected in stained smears of the solution and also that the presence of Merthiolate as a preservative did not change the rate of fall of viscosity.

Lusena (2) and Lusena and Adams (3) have recently published methods for obtaining dry gluten preparations by freeze- or spray-drying acetic acid dispersions of the gluten. The adoption of the heat-treatment step in their procedures would result in more uniform products, insofar as the magnitude of degradative changes during subsequent handling would be diminished.

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August 14, 1950

BOOK REVIEWS

A German-English Dictionary for Chemists, 3rd edition, by Austin M. Patterson. 541 pp. John Wiley and Sons, Inc., New York, Chapman and Hall, Ltd., London. Price $5.00.

This book is designed chiefly for chemists and chemical engineers but it is also useful for workers in other fields of science such as physics, biology and geology. Compared with the second edition which had 42,000 entries, this third edition has 59,000. The increase has resulted partly from additional meanings to words listed in the former edition, but chiefly from new additions in the fields of chemical technology, electronics and warfare. Still carrying a good vocabulary of general words, this dictionary, which is well-printed, serves as a great stand-by for all except the experts in the language who are interested in German scientific literature.

F. Smith

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August 3, 1950

"A Textbook of Biochemistry." By Philip H. Mitchell. 2nd edition, 1950. McGraw-Hill Book Co., Inc. $6.00. xvii + 695 pp.

The second edition of this generally well-accepted book retains the arrangement and sequence of subject matter used in the first edition published in 1946. The 21 chapters of the book deal with carbohydrates, photosynthesis, fats and related substances, proteins and amino acids, nucleoproteins and nucleic acids, the vitamins, enzymes, digestion, absorption and detoxication, blood and lymph, chemistry of respiration and acid-base balance, biological oxidation, calorimetry and energy metabolism, carbohydrate metabolism, metabolism of lipids, protein metabolism, urine, composition of foods: dietetics, the chemistry of animal tissues, chemistry of the hormones, and chemotherapy. As might be gathered from the table of contents, the subject of biochemistry in this book is treated almost exclusively from the standpoint of animal biochemistry and the chapter on photosynthesis appears to be almost superfluous in this context. The second edition covers 55 pages more than the first but this enlargement is accounted for in part by the greater marginal spaces on each page and the expansion in text is partly compensated by a regrettable abbreviation of the subject index.

According to the author "Emphasis in this text has been given and major space allotted to such subjects as the constitution and activities of enzymes, the intermediary reactions of anabolism and catabolism and the vital significance of hormones and vitamins." In spite of this avowed purpose of the book, most of the material in the second edition has not been greatly changed to reflect the tremendous research activity and progress which have taken place in the four year interval between the first and second edition. Thus, the highly significant study of localization of enzymatic and other cellular constituents in such particles as mitochondria, microsomes and nuclei appears to be overlooked. The paragraph on page 660 on the outlook in chemotherapy remains essentially unchanged from the first edition in spite of the great advances which have been made in this field. The treatment of the present concept of high energy phosphate bonds is extremely sketchy and the reactions concerned in their regeneration are almost completely neglected. The reaction between phosphopyruvic acid and ADP (p. 441) is still pictured as being irreversible. In the second edition many errors occurring in the first edition have been eliminated while others unfortunately appear unchanged. Thus, "Dimedon" is erroneously referred to on page 55 as "dimethylhydroxyresorcinol"; the Reichert Meissl number is described as a measure of the "volatile fatty acid content of a fat" and decanoic acid is included among the acids contributing to it (p. 77). On page 365 the carbohydrate component of coenzyme I is written with the formula of D-lyxose but is labeled as D-ribose.

A very admirable feature of Dr. Mitchell's book is the numerous references to review articles and to research papers given at the end of each chapter, but here, too, the reviewer feels that many of the older references could have profitably been replaced by those which would guide the student to the more recent work. In spite of the shortcomings which have been pointed out, Dr. Mitchell's book is particularly suitable for use by nonmedical students of biochemistry. The reviewer, for instance, will continue to recommend its use for supplementary reading to a class of undergraduate students taking a course in "animal biochemistry."

The physical makeup of the book and the binding are good and few typographical errors have been noted.

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September 28, 1950

ERRATA

Cereal Chemistry, Vol. 27, No. 5

(September, 1950)

Page 370. In revising their manuscript, Irvine *et al.* made certain changes in Table II, page 371, but unintentionally overlooked alterations in the text in which these data are discussed. Paragraph two should read:

> "Table II lists data for the mixing reaction. The rate of the mixing reaction is largely independent of pigment level and varies, less widely than the rate of the initial reaction for the samples studied, from 0.300 to 0.501 p.p.m. per minute, a factor of slightly less than two. Cyanide inhibits the rate of mixing reaction for four of the six varieties as much as 53%; over-all inhibition of the reaction to ten minutes mixing by cyanide is marked for all of the varieties tested, and varies from 27 to 52%. Inhibition of the mixing reaction by 30% alcohol is very marked in all cases, ranging from 70 to 100%, and the net balance between acceleration of the initial reaction and inhibition of the mixing reaction after ten minutes of mixing shows an over-all inhibition in all cases, varying from 17 to 41%.

Please make the following corrections in the paper by Fifield *et al.*:

Page 388. Line 11, for "17" read "77."

Page 389. Line 9, for "load" read "loaf."

Cereal Chemistry

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SUGGESTIONS TO AUTHORS

General. From January 1, 1948, an abstract will be printed at the beginning of each paper instead of a summary at the end, references will be numbered to provide the option of citing by number only, and date of receipt, author's connections, etc., will be shown in footnotes. Except on these points, authors will find the last volume of *Cereal Chemistry* a useful guide to acceptable arrangements and styling of papers. "On Writing Scientific Papers for Cereal Chemistry" (*Trans. Am. Assoc. Cereal Chem.* **6**: 1–22. 1948) amplifies the following notes.

Authors should submit two copies of the manuscript, typed double spaced with wide margins on 8½ by 11 inch white paper, and all original drawings or photographs for figures. If possible, one set of photographs of figures should also be submitted. Originals can then be held to prevent damage, and the photographs can be sent to reviewers.

Titles and Footnotes. Titles should be specific, but should be kept short by deleting unnecessary words. The title footnote shows "Manuscript received . . ." and the name and address of the author's institution. Author footnotes, showing position and connections, are desirable although not obligatory.

Abstract. A concise abstract of about 200 words follows title and authors. It should state the principal results and conclusions, and should contain, largely by inference, adequate information on the scope and design of the investigation.

Literature. In general, only recent papers need be listed, and these can often be cited more advantageously throughout the text than in the introduction. Long introductory reviews should be avoided, especially when a recent review in another paper or in a monograph can be cited instead.

References are arranged and numbered in alphabetical order of authors' names and show author, title, journal, volume, first and last pages, and year. The list is given at the end of the paper. Reference numbers must invariably be cited in the text, but authors' names and year may be cited also. Abbreviations for the names of journals follow the list given in *Chemical Abstracts* **40**: I–CCIX. 1946.

Organization. The standard organization involves main sections for abstract, introduction, materials, methods, results, discussion, acknowledgments, and literature cited. Alternately, a group of related studies, each made with different materials or methods, may require a separate section for each study, with subsections for materials and methods, and for results, under each section. Center headings are used for main sections and italicized run-in headings for subsections, and headings should be restricted to these two types only.

Tables. Data should be arranged to facilitate the comparisons readers must make. Tables should be kept small by breaking up large ones if this is feasible. Only about eight columns of tabular matter can be printed across the page. Authors should omit all unessential data such as laboratory numbers, columns of data that show no significant variation, and any data not discussed in the text. A text reference can frequently be substituted for columns containing only a few data. The number of significant figures should be minimized. Box and side heading should be kept short by abbreviating freely; unorthodox abbreviations may be explained in footnotes, but unnecessary footnotes should be avoided. Leader tables without a number, main heading, or ruled lines are often useful for small groups of data.

Tables should be typed on separate pages at the end of the manuscript, and their position should be indicated to the printer by typing "(TABLE I)" in the appropriate place between lines of the text. (Figures are treated in the same way.)

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All drawings should be made about two to three times eventual reduced size with India ink on white paper, tracing linen, or *blue*-lined graph paper; with any other color, the unsightly mass of small grid lines is reproduced in the cut. Lettering should be done with a guide using India ink; and letters should be $\frac{1}{16}$ to $\frac{1}{8}$th inch high after reduction.

For difficult photographs, a professional should be hired or aid obtained from a good amateur. The subject should be lighted to show details. A bright print with considerable contrast reproduces best, and all prints should be made on glossy paper.

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Text. Clarity and conciseness are the prime essentials of a good scientific style. Proper grouping of related information and thoughts within paragraphs, selection of logical sequences for paragraphs and for sentences within paragraphs, and a skillful use of headings and topic sentences are the greatest aids to clarity. Clear phrasing is simplified by writing short sentences, using direct statements and active verbs, and preferring the concrete to the abstract, the specific to the general, and the definite to the vague. Trite circumlocutions and useless modifiers are the main causes of verbosity; they should be removed by repeated editing of drafts.

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Use names, not formulas, for text references to chemical compounds. Use plural verbs with quantities (6.9 g. were). Figures are used before unit abbreviations (3 ml.), and % rather than "per cent" is used following figures. All units are abbreviated and followed by periods, except units of time, which are spelled out. Repeat the degree sign (5°–10° C.). Place 0 before the decimal point for correlation coefficients ($r = 0.95$). Use * to mark statistics that exceed the 5% level and ** for those that exceed the 1% level; footnotes explaining this convention are no longer required. Type fractions on one line if possible, e.g., $A/(B + C)$. Use lower case for farinograph, mixogram, etc., unless used with a proper name, i.e., Brabender Farinograph. When in doubt about a point that occurs frequently, consult the Style Manual or the Dictionary.

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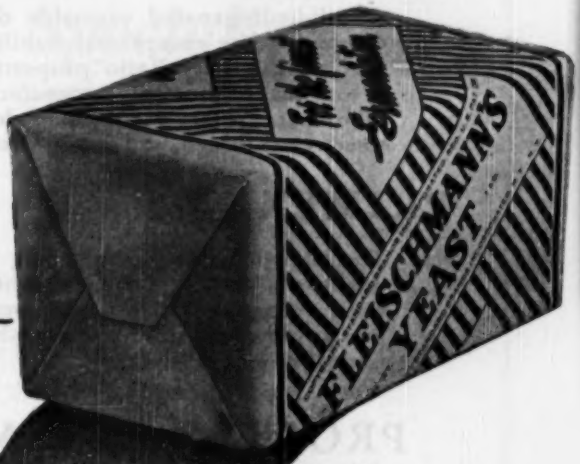

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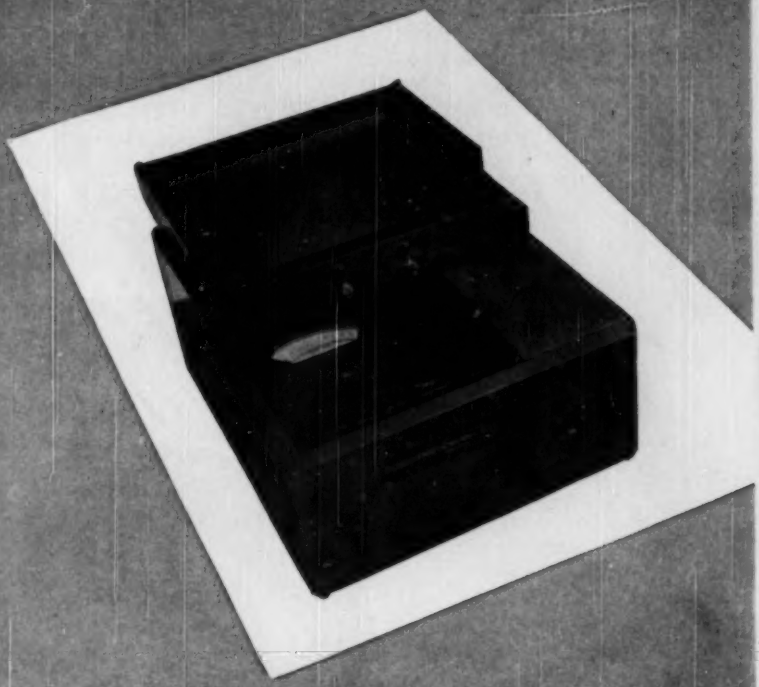

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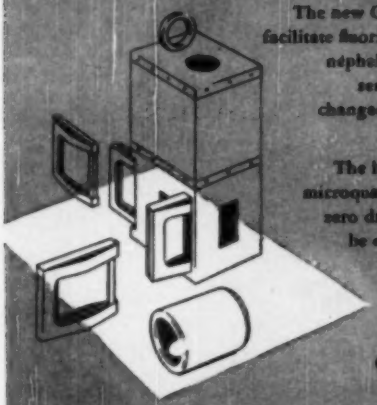

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Service When It Counts

Every so often, we all need assistance and need it badly, to furnish the answers to many of our problems. In the Flour and Cereal Industry, N-A's Flour Service Division has been supplying practical, down-to-earth solutions to many of the industry's problems for over 25 years. Using its broad background of experience in the manufacture of time-tested products for maturing, bleaching, and enriching, the N-A Flour Service Division is ready to put its nation-wide service organization and extensive laboratory and research facilities at your service. They will work closely with your own staff and consultants to produce a concrete, individual solution to fit your particular needs.

For more details, call your nearest N-A Representative today and find out how this service can work profitably for you.

WALLACE & TIERNAN COMPANY, INC., AGENTS FOR

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